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EFFECT OF SALTS OF HEAVY METALS ON DEVELOPMENT OF THE SEA URCHIN, ARBACIA PUNCTULATA

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I

In previous experiments an examination has been made of the effects on gastrulation and embryonic differentiation in *Arbacia* of ether, changes in temperature, alcohols, X-rays, hypertonic and hypotonic sea water, and of the disturbance of the salt balance in sea water. Disturbance of the salt balance of sea water and the action of certain alkaline earth metals etc., have been tested on the sea urchin (*Paracentrotus lividus*) by Runnström (1935), Lindahl (1936), Lindahl and Stordal (1937) and others in relation to gastrulation, ectodermization and endodermization of the embryo and specifically to differentiation. References to other workers have been given in earlier papers. These studies have been concerned with the unfertilized and fertilized egg rather than with the blastula stage.

At gastrulation physical movements take place that result in an invaginated endodermal tube from which arises much of the mesodermal material. These movements may be delayed temporarily, retarded or inhibited, and they also may be influenced independently of differentiation. In the latter case an exogastrula may or may not form. What initiates the movements is another problem, but at least it has been shown that a host of factors, producing quite similar effects, can interfere with gastrulation; disturb the proportions of potential ectoderm and endoderm; and influence the differentiation of skeleton, body form, ectoderm and endoderm.

As regards embryonic differentiation, the effect has been in general very much the same for these various environmental modifications. Some types of differentiation, taking place as development proceeds, can be slowed down to a greater extent than others. Ectoderm, and to a lesser extent mesoderm, may grow and differentiate when endoderm formation is entirely inhibited. Furthermore, the ectoderm is

the most resistant to environmental modifications. Different cultures may vary under apparently identical conditions; cultures from one female may vary from those of another; while individuals in the same culture always vary in their susceptibility. The exact condition of early development, i.e. fertilized egg to early gastrulation, seems of minor importance, since the results are somewhat similar under the conditions of the experiments.

Some metallic salts, which are a natural constituent of sea water, are more toxic to animal life than are others. On the whole, it is probable that the concentration of a particular metal changes very slightly throughout the ocean. The possible exception may be where large bodies of water empty into the ocean causing dilutions of certain constituents of sea water and concentrations of others, especially metals. What concentrations can the developmental stage of a littoral animal such as the sea urchin, *Arbacia punctulata*, withstand and what is the effect of increased concentration of different metals on such a developmental process as gastrulation? Finally, are the effects on development comparable to those secured by other experimental methods?

II

In this study $ZnCl_2$, $ZnSO_4$, $Zn(C_2H_3O_2)_2$, $FeCl_2$, $PbCl_2$, $CuCl_2$, $HgCl_2$, $AlCl_3$, $NiCl_2$ and $CdCl_2$ have been used in various concentrations. As in previous studies, the blastula stage was selected for study and exposure was made for long periods of time.

Reference to Tables I–IV will show the approximate concentrations which produced certain results. The concentrations are expressed as proportions and represent the amount of the metallic salt which was added to the sea water. No estimate was made of the normal amount of the metal in sea water. As nearly equal numbers of embryos as possible, without actually counting them, were used in each culture. By mixing the embryos secured from several females more or less similar cultures were secured. All results were based on random sampling from three places in the culture. The exposure time was varied, and in certain cases where developmental arrest had occurred without death, the culture was washed and returned to fresh sea water for recovery. In some instances samples were transferred at intervals from a lethal solution to fresh sea water to study the rate of the toxic action and the recovery ability of the embryos.

It is said that many of the heavy metal salts are precipitated by sea water. The concentrations specified in this study may represent initial rather than final concentrations and some of them may have changed in the course of the experiment. At this point it may be

said that those experiments were discarded in which visible precipitation had occurred. The whole subject of heavy metal action is obscure.

This study was made at room temperature during the months of July and early August. Hence there was some variation as some of the experiments ran for several days. To avoid repetition only the results secured from the exposure to $ZnCl_2$ will be described, but comparisons with the other metals will be made. The data for the others are included in the tables.

III

A survey of the more recent literature has revealed numerous references to the effects of heavy metal salts on animal development and embryonic differentiation. The effect on gastrulation has apparently not been tested.

Hammett and Wallace (1928) found that the lead ion retarded growth, and differential development of the head and optic regions was markedly inhibited in chick embryos. Child (1929) has used $CuSO_4$ in the study of physiological gradients in the chick embryo. Féré (1893) obtained monsters following the injection of lead nitrate into developing chick embryos (see also Franke, 1936, for selenium salts).

Galtsoff (1932) has shown that marine invertebrate animals can concentrate different metallic elements in their bodies. Certain groups concentrate zinc, others copper, etc. Copper salts affect oyster larvæ by inducing attachment (see also Prytherch, 1931), and by initiating metamorphosis. Metallic silver causes sperm of the sea urchin to lose their fertilizing power, and paralyses plutei (Drzewina and Bohn, 1926).

Hoadley (1923) has studied the effects of the salts of the heavy metals on the fertilization reaction in the sea urchin, *Arbacia*. The inhibiting concentration varied for the different metals tested. Gold chloride was most toxic for membrane elevation and cleavage and cadmium or cobalt chloride least. Other salts tested included $CuCl_2$, $ZnCl_2$, $LaCl_3$, $AlCl_3$, $PtCl_4$, $PbCl_2$, $NiCl_2$ in the order of the toxicity. $HgCl_2$ differed from the others in that membranes elevated at a concentration of 1 part in 600,000, which was toxic for cleavage. Concentration of these metals varied slightly for different batches of eggs and thus showed the influence of a time factor.

$HgCl_2$ has a harmful effect on cleavage at different concentrations following very short exposures (Hoadley, 1930). It affects the cortical region resulting in membrane elevation. After longer exposures it affects the pigment which has mercury-avid properties. The pigment

may be extruded and such an egg may develop if not injured. A conceivable mechanism is thus available by which the mercuric ion, which has entered the egg, may be bound and removed.

Copper salts are known to have an injurious effect on many organisms. In a very low concentration which inhibits fertilization, sperm may be still active. Inhibition is marked in a concentration as low as 1 part in 2,500,000 parts of sea water and is reversible provided the eggs are not injured too much. Copper appears to injure the vitality of eggs and acts as a slow poison (Lillie, 1921). A concentration of 1/62,500 is necessary to suppress cleavage. The effect of $HgCl_2$ is different from that of copper: initial stages of fertilization are little affected, susceptibility increases as fertilization progresses, fertilized eggs show the effect more rapidly than unfertilized eggs, and the movement of sperm is suppressed at great dilutions. It produces membrane elevation alone and favors it in fertilization. The effects are reversible if exposure is not continued too long. Lillie concludes from these studies that the effect of mercury and copper on fertilization following membrane formation may be due to enzyme poisoning. The effect on the initial stages of the fertilization reaction does not correspond so well to the enzyme analogy.

Glaser (1923) shows that in the egg of *Arbacia* copper becomes concentrated in the chorion, vitelline membrane and cortex. It is diffused throughout the cytoplasm. Copper occurs normally in egg pigment, membrane chorion and cortex associated with lipolysin. Normal eggs secrete copper compounds as well as removing copper sulphate from sea water. Parker (1924) has shown that marine animals will grow upon any heavy metal plate provided the metal does not liberate ions or soluble compounds. The effect of $CuCl_2$, $MnCl_2$, and $FeCl_3$ upon cardiac explants cultured in vitro has been studied by Hetherington and Shipp (1935). The survival time was tested. Other interesting studies could be given but the above are sufficient to indicate the type of work which has been done.

IV

$NiCl_2$.—In an experiment to test the effect of this metallic chloride upon the development and differentiation of the germ layers after their formation had been initiated, oval blastulæ to early gastrulæ were exposed to various dilutions of a 1 per cent stock solution made up in sea water. The cultures were examined after 21–42 hours of exposure and in some instances the embryos were returned to fresh sea water. $NiCl_2$ gave the best and most numerous examples of exogastrulation of all the metallic salts which were tried. Further-

more, the exogastrulæ underwent further differentiation. The lowest concentrations employed gave also marked inhibition of development.

After 21 hours exposure in a concentration of 1 part in 60,000, development had only progressed to a large triangular stage with skeletal spicules or short rods and initial flap formation. A few simple exogastrulæ with tri-radiate spicules were seen in the culture. During the same length of time the control embryos had advanced to a medium pluteus stage. A 42-hour exposure killed most of the experimental embryos although a few large blastulae and triangular stages showed slow movement.

In concentrations of 1/40,000, 1/20,000, 1/14,285, development was progressively inhibited but the number of exogastrulæ increased markedly. The embryos were all on the bottom of the culture dish. Both the evaginated and invaginated endodermal tubes failed to differentiate although some growth occurred, while the mesenchyme either formed small spicules or failed to show any skeleton-forming activity. The ectoderm was the least affected and continued growth. It acquired its characteristic appearance and the apical plate appeared. In such cases where the formation of the endoderm was entirely inhibited, large circular or oval ectoblastulæ were seen. A longer exposure killed the embryos, while those which had been transferred to fresh sea water showed increased activity but only slightly advanced development, especially in the case of the ectoderm and skeleton. The endoderm of these embryos showed no further change. The ectoderm in the region of the vegetal pole tended to be more or less irregular and the shape of the body was often lumpy.

When transferred to fresh sea water after 21 hours exposure, the embryos either died or failed for the most part to continue development. The activity of the survivors increased. Blastulæ and exogastrulæ with spicules, stomodæum and apical plate were seen. Some of the survivors showed no formation of endoderm while others contained a simple endodermal tube but no skeleton. Thus the embryos were unable to overcome in sea water the poisonous effects of the metal. It acts in very low concentrations (Table I) and within a relatively short time. The effects are not reversible and embryos in a culture react differently. The various abnormal types seen in the cultures are the result of differential susceptibility among the embryos and also in the developmental processes involved. This has been a common observation in previous studies of gastrulation in the embryos of *Arbacia*.

The number of exogastrulæ decreased in concentrations from 1/10,000 to 1/6,000. Differentiation was further inhibited while

TABLE I

Effect of different concentrations of $NiCl_2$ on gastrulation and embryonic differentiation.

Control	1/60,000	1/40,000	1/20,000	1/14,285	1/10,000	1/6,666	1/5,000	1/2,857
			Oval to Spherical Blastulæ					
Gastrulæ to medium plutei	Gastrulæ to triangulars with spicules	Medium to late gastrulæ	Blastulæ to late gastrulæ. No skeleton.	More blastulæ. Movement slower. Few large simple exos.	Fewer attempts at gastrulation. Many larger simple exos, or irregular endoderm formation.	Few initial gastrulæ. Many slight exos.	No gastrulation. Few exos.	All alive. No change.
			Late Blastulæ to Initial Gastrulæ					
Medium plutei	Large triangular with skeleton. Some abnormalities. Few exos.	Somewhat smaller. Most on bottom. Few exos.	All on bottom. Blastulæ vesiculars. More exos. Few initial gastrulæ.	Blastulæ to simple gastrulæ. Few with spicules. Many exos.	Small blastulæ. Many exos. Spicules in the above.	Small blastulæ. Fewer exos. No spicules.	Blastulæ to triangular with spicules. Few exos.	All alive. No change.

growth and movement became slower. The ectoderm was the most resistant. In a concentration of 1 part in 5,000, there occurred some increase in size but no development. After 21 hours in a 1/2,000 concentration there were few intact individuals left which had not progressed beyond the state at which they were placed in the solutions.

In an effort to see how quickly the embryos would be affected, samples from a series of different concentrations were transferred at certain intervals to fresh sea water. These were examined about 24 hours later. The results are summarized in Tables II and III. They show that a relatively strong concentration of $NiCl_2$ which inhibits any further development in the experimental solution acts very quickly upon the embryo. For example, 6–7 hours exposure to a 1/2,000 concentration was fatal to further differentiation but the embryos lived and increased in size (growth) in fresh sea water.

In the tables, comparative data are given for concentrations ranging from 1/10,000 to 1/1,666. In a 1/2,000 concentration endoderm formation was entirely inhibited following an exposure of about 6 hours. In a 1/10,000 concentration the gastrulation processes first showed inhibitory effects after 7 hours in some individuals of a culture. Up to this time, the effect had been upon differentiation and growth. In several repetitions of this experiment, a peculiar result was seen. After 3–4 hours exposure followed by transfer to fresh sea water for 18–19 hours, quite normal appearing embryos were found. Longer exposure times likewise gave more active and better differentiated embryos than did the shorter exposures.

This result is surprising since it was expected that continued exposure would eventually result in death for all individuals. Is it possible that over a long exposure period the embryos lost their sensitivity to the toxic effect of the salt and were able to differentiate normally or that the salt was taken out of solution in some way? This result was observed in three out of the five repetitions. In a 1/5,000 concentration development was progressively inhibited up to about $4\frac{1}{2}$ hours while after $6\frac{1}{2}$ hours there was a marked advance in differentiation. In a 1/2,857 concentration this "pick up" was observed after $4\frac{1}{2}$ hours exposure. In a 1/2,000 concentration the improvement was noticed in cultures exposed to the salt for $3\frac{1}{4}$ hours or longer.

In other experiments, Tables II and III, the above phenomenon was not observed. These show a progressive depression of development in the higher concentrations. In Table III it can be seen that the capacity to gastrulate was destroyed in a 1/5,000 concentration after about 8 hours exposure, and no recovery was made in fresh sea

TABLE II

Effect of exposure of blastulæ to different concentrations of $NiCl_2$ for varied periods of time. Blastulæ placed in solution and samples removed at intervals to fresh sea water. Examined 18–19 hours later. At this time the control was at a medium pluteus stage. The data show the rate and degree of toxic effect.

Solution	Hour intervals when change was made					
	1	3	4	5	6	7
1/10,000	Like control	Some retardation. Also vesicular with gut and skeleton to some with initial arm flaps.	Less retardation. Body larger and arms longer.	Stunted forms to young plutei with abnormal skeleton	All types up to young plutei; with short body and widely spaced arms.	All types up to young plutei. Gastrulæ with gut and spicules. Ectoderm lumpy in some cases.
1/3,333	Young plutei. Some with short arms or arm flaps.	Large triangular embryos with gut. Skeleton often irregular.	Large spherical blastulæ to triangular gastrulæ with spicules. Few simple exogastrulæ.	More blastulæ and fewer gastrulæ. Fewer of the latter have reached the triangular stage.	Many large, vesicular embryos. Few with flattened or irregular vegetal pole region. Few small triangulars and gastrulæ with gut and spicules.	Most are large and small vesicular embryos. More triangulars with gut, skeleton spicules. Short body.
1/2,500	Large, triangular. Skeleton often abnormal.	Same	Vesicular forms with gut	Irregular triangular with spicules. Some are vesicular with no gut.	Vesiculars. Few have gut.	Almost all on bottom. Vesiculars no gut.

TABLE II—*Continued*

Solution	Hour intervals when change was made					
	1	3	4	5	6	7
1/2,000	Large triangular embryos with gut and normal or irregular spicules. Some showed initial arm-flap formation. Ectoderm ragged in many. Body flattened.	Smaller. More vesicular. Large and small spicules. Some showed initial arm-flap formation.	Smaller and more vesicular gastrulæ. Small spciules. No initial arm flaps.	All small in size. Vesicular and irregular in outline. No skeleton. Fewer gastrulæ.	Embryos irregularly vesicular. Most show no endoderm tube, or mass of cells, or external irregularities.	Most all on bottom of dish. No internal gut. Vegetal pole region irregular in outline. A few undifferentiated and irregularly shaped exogastrula.
1/1,666	Small late gastrulæ with spicules	Small blastulæ to triangular gastrulæ with spicules. Few simple exogastrulæ.	Blastulæ to medium gastrulæ, no spicules	Mostly blastulæ. Very few showed initial gastrulation.	All blastulæ	Some showed disintegration

TABLE III

Effect of exposure of blastulæ to different concentrations of $NiCl_2$ for varied periods of time. Spherical to oval blastulæ put in solutions at a certain time and then removed to fresh sea water at intervals thereafter. These cultures examined about 24 hours later. Control showed plutei. The data show the rate and degree of the toxic effect.

Solution	Those left in in solution	Hour intervals when change was made to fresh sea water							
		2	3	4½	6	7½	9	10½	12
1/10,000	Plutei like controls	Triangular to small plutei	Same	Gastrulæ to triangular with skeleton	Small triangular with spicules	Vesiculars to gastrula	Good plutei	Same	Like control
1/5,000	Blastula to initial gastrula. Little movement	Irregular gastrula to small triangular with skeleton	Some more retardation. Spicules or none	Blastulæ to vesicular and gastrulæ. Few triangular with spicules.	Most on bottom. Irregular and lumpy in outline. More blastulæ. No skeleton.	All on bottom. More vesicular. Fewer gastrulæ.	Most are spherical blastulæ	Most are spherical blastulæ	More vesicular
1/3,333	Blastulæ to triangular gastrulæ all on bottom	Small young plutei	Vesicular to triangular with spicules. Few exos.	Same. Fewer on top	More blastulæ to large vesiculars	None on top. Blastulæ to initial gastrulæ.	Same	Same	Spherical to oval blastulæ

TABLE III—*Continued*

Solution	Those left in solution	Hour intervals when change was made to fresh sea water							
		2	3	4½	6	7½	9	10½	12
1/2,500	Spherical to oval blastulæ	Late gastrula to triangular with spicules	Many on bottom. Blastulæ to late gastrulæ with spicules.	Same. All on bottom.	Blastulæ to late gastrulæ. No spicule.	Blastulæ to slight gastrulæ	Same	Blastulæ	Same
1/2,000	Blastulæ	Blastulæ to late triangular with skeleton	Same. No skeleton.	All on bottom. Some disintegration.	Same	Blastulæ	Same	Same	Same. Some disintegration.
1/1,666	Blastulæ. Some disintegration.	Most on bottom. Blastulæ to triangular with skeleton.	Same	All on bottom. More blastulæ or slight invagination.	Blastulæ. Few initial invagination.	Same	About same	Blastulæ of various shapes	Same

TABLE IV

Effect upon development of the addition of various amounts of different metallic salts to those already present in sea water.

Metal	Control	Length of exposure	1/66,666	1/40,000	1/20,000	1/14,285	1/10,000	1/8,333	1/6.666	1/6,000	1/5,000
Al_2Cl_6 1.0%	Young plutei	13½ hrs.	Gastrula to young plutei	About same	Blastulæ to very late gastrulæ	Blastulæ to initial gastrulæ. Few top swimmers.	Fewer initial gastrulæ	Mostly blastulæ. Occasional initial gastrula.	No gastrulation. Spherical masses of cells.	Same	Some disintegration
$CdCl_2$ 1.0%	Young plutei	13½ hrs.	Gastrulæ to triangulars with spicules	Gastrulæ to early triangulars. No spicules.	Young to late gastrulæ	Blastulæ to medium gastrulæ	Occasional initial gastrulation	Young to late blastulæ	Same. Few swimmers.	Same. Some membranes persist.	Same. All in bottom. Disintegration.
				1/400,000	1/200,000	1/142,850	1/100,000	1/83,333	1/66,666	1/60,000	1/50,000
$PbCl_2$ 0.1%	Young plutei	13½ hrs.		Like control		Like control	Slight retardation	Gastrulæ to triangulars	Young to late gastrulæ	Blastulæ to medium gastrulæ	More blastulæ and fewer gastrula
$FeCl_3$ 0.1%	Young plutei	13½ hrs.	Practically no difference from the control								
			1/3,200,000		1/1,600,000	1/800,000	1/500,000	1/400,000	1/300,000	1/250,000	1/200,000
$CuCl_2$ 0.1%	Young plutei	16 hrs.	Like control		Gastrulæ to triangulars. Spicules.	Young to late gastrulæ	Initial to young gastrulæ. Some membranes persisting. Many in bottom.	Blastulæ to initial gastrulæ. Many still in membranes. Most on bottom.	Blastulæ only. Many in membranes. All on bottom.	Blastulæ only. Some disintegration.	Same. More disintegration.
$ZnCl_2$ 0.1%	Young plutei	16 hrs.	Like control		Gastrulæ to large triangulars	Late gastrulæ	Much the same. Slower movement.	Medium gastrulæ. Most on bottom.	Blastulæ to young gastrulæ	More blastulæ. Initial gastrulation.	Mostly blastulæ. Empty membranes persist.
$(ZnSO_4)$ 0.1%	Young plutei	15 hrs.	Like control		Like control	Slight retardation	Triangulars	Gastrulæ	Same	Blastulæ to young gastrulæ. Some dead.	About same
0.1% $Zn(C_2H_3O_2)_2$	Young plutei	17 hrs.	Like control		Slight retardation	Gastrulæ to young plutei	Gastrulæ	Blastulæ to young gastrulæ. Membranes persisting.	Some	Blastulæ to initial gastrulæ	About same. Large blastopore.
			1/48,000,000		1/32,000,000	1/16,000,000	1/8,000,000	1/5,000,000			
$HgCl_2$ 0.01%	Young plutei	15 hrs.	Early gastrula to triangulars without skeleton		Blastulæ to medium gastrulæ	Blastulæ to young gastrulæ	Blastulæ. Much disintegration.	Dead. Masses of spherical cells.			

water. The length of time necessary to destroy the ability to gastrulate varies according to the concentration employed; Tables II and III. The results summarized in the tables represent single experiments, but they are indicative of what was secured by several repetitions of each.

Al_2Cl_6.—In dilutions of a 1 per cent stock solution of Al_2Cl_6, gastrulation in all individuals was practically inhibited at a 1/8,333 concentration during an exposure of 13½ hours. Table IV shows that the first indication of the inhibition of gastrulation in some of the individuals was observed at a concentration of 1/20,000. Further inhibition of more individuals occurred in higher concentrations until at a 1/6,666 concentration no gastrulation took place during the experimental period. Samples transferred to fresh sea water from concentrations between 1/14,285 to 1/8,333 after this interval recovered. Gastrulation and development to the pluteus stage took place. Abnormalities of arm and skeleton formation appeared in the sample from the 1/8,333 culture. Samples transferred to sea water from the 1/6,666 culture died.

To test the rapidity of the toxic effect, samples were transferred from a 1/6,666 concentration, at certain intervals, to sea water. Those transferred after ½ to 2½ hours of exposure gave plutei. The longer exposures caused progressive retardation, and more gastrulæ and fewer plutei were found for the same length of time. After 5 hours exposure, a few gastrulated but most died in the sea water; after 6½ hours exposure, all died without any development.

When the toxic effect of a 1/5,000 concentration was tested, an exposure of ½ hour markedly retarded development, 1 hour inhibited almost all gastrulation and 2½ hours killed the embryos.

$CdCl_2$.—Table IV includes the effect of this metallic chloride in different concentrations. Over a 13½ hour period, gastrulation was inhibited in a 1/8,333 concentration. In sea water, these blastulæ grew in size, became irregular in outline, but did not gastrulate or form skeleton. Samples from a 1/10,000 concentration formed large, vesicular, irregular blastulæ and gastrulæ in sea water.

$PbCl_2$.—Lead chloride is slightly more toxic than the three metallic chlorides just mentioned, and hence a 0.1 per cent stock solution was used. Table IV includes part of the data secured in one experiment which may be considered typical. Slight retardation became evident in a concentration of 1 part in 100,000. In a concentration of 1/60,000 gastrulation was inhibited in some individuals. The latter increased in number in a concentration of 1/50,000 after which little change was noted in the cultures until a concentration of 1/35,714 was reached.

One result was the persistence of the fertilization membranes even after the embryos had escaped. Thus this salt causes the membranes to harden and prevents their dissolution during the experimental period.

In the 1/35,714 solution late gastrula to triangular stages were found, while in a 1/33,333 concentration even young plutei occurred. This condition was found in all cultures up to a concentration of 1/22,222. No higher concentrations were tried. This peculiar change may perhaps be accounted for by the precipitation of lead seen in the bottom of the container, during the experimental period. The precipitation did not appear when the experimental solutions were made up, but only after standing. Enough was left in solution to retard development slightly.

$FeCl_2$.—This metallic chloride is apparently not toxic to any extent. Very little if any difference was observed in graded cultures up to a concentration of 1 part in 14,285.

$CuCl_2$.—This metallic salt is far more toxic for development than $PbCl_2$. A 0.1 per cent stock solution was used. Table IV summarizes the results of a typical experiment. Gastrulation was inhibited in most individuals in a concentration of one part in 400,000 and entirely in a 1/300,000 concentration. The fertilization membranes showed a hardening in these concentrations and failed to dissolve. This must occur rather quickly because in these and higher concentrations more of the embryos failed to escape from the membranes. The same persistence of the membranes was also seen in the $PbCl_2$ solutions but to a lesser extent. In the latter case the embryos had escaped so the hardening occurred more slowly. This lack of digestion of the membranes in both the $PbCl_2$ and $CuCl_2$ solutions may be accounted for by enzyme-poisoning which is characteristic of some of the heavy metal salts. As will be seen below, the higher concentrations of the Zn salts likewise produced persistent membranes.

After transfer to fresh sea water, the original 1/500,000 culture continued development to the pluteus stage. Also large vesicular blastulæ without skeleton were found, indicating growth of the ectoderm but inhibition of endoderm and mesoderm formation. Most of the individuals in the 1/400,000 concentration gastrulated in fresh sea water and in 24 hours formed triangular embryos with spicules. More large blastulæ were present as well as a few exogastrulæ showing tri-partite gut, anal opening, and apical plate. Thus in some individuals this metallic salt inhibits the gastrulation processes without inhibiting endoderm formation.

As seen in Table IV, no gastrulation occurred in a 1/300,000

concentration during the experimental period. However, in sea water some did gastrulate and a very few formed simple exogastrulæ. No skeleton was seen in any individual, indicating the inhibition of mesoderm formation or the formation of skeleton during the period of observation. The endoderm did not differentiate. Only a few individuals from a 1/250,000 concentration recovered in sea water. Large, spherical and oval blastulæ were found as well as a few initial gastrulæ. The individuals from a 1/200,000 culture failed to gastrulate even after 48 hours; many died; and many failed to get out of the membranes.

The time required for recovery varies of course with the concentration. Embryos which did not gastrulate or in which no skeleton formed during a 24-hour period in sea water, did so after 48 hours. Since it is known that certain marine organisms may store copper in their tissues (oysters) this inhibition may be due to the concentration of the toxic metal, and the slowness of recovery and differentiation be due to the slowness with which the metal left the cells and again reëntered the sea water in such a dilute concentration as not to inhibit further development. The metal could not have irreversibly injured the protoplasm, but rather inhibited its function.

Angerer (1935) has found that after *Arbacia* eggs were exposed to $CuCl_2$ an interval of time elapsed during which the metal produced no visible effect in the protoplasm as regards its viscosity. This time interval is a function of the concentration of copper in solution. At the conclusion of this latent period, there ensues a sharp rise in viscosity values resulting in an irreversible gelation (coagulation) of the protoplasm. In the case of *Arbacia* egg protoplasm, there is no concentration at which gelation is reversible.

In the light of Angerer's results the temporary inhibition of differentiation cannot have been due to gelation, otherwise recovery would not have occurred. An irreversible effect was seen first in the concentration of 1/200,000 where no recovery occurred even during 48 hours in sea water.

To test the rate of lethal action of $CuCl_2$, a concentration of 1 part of stock solution in 150,000 parts sea water was used, which inhibits all development and causes complete disintegration in an 18-hour period of exposure. Embryos were removed from this solution to fresh sea water at various intervals. An exposure of $3\frac{1}{2}$ hours resulted in some inhibition and many persistent membranes from which numerous embryos had failed to escape. After $9\frac{1}{2}$ hours in the solution only a few embryos gastrulated during the following 28 hours in sea water. Those which did not escape from the membranes, or were only

partially successful in it, did not differentiate. A concentration of 1/250,000 produced no inhibitory effects during 8 hours exposure. Membranes hardened while the embryos which did not escape differentiated slightly.

$HgCl_2$.—This metallic chloride is very toxic. A concentration of 1 part in 48,000,000 parts of sea water retarded development while a concentration of 1/8,000,000 prevented gastrulation during a 15-hour exposure (Table IV). After return to sea water, only very few of the more hardy individuals were able to gastrulate, but they developed no further. In a concentration of 1/5,000,000, the swollen cells showed a clumping of the pigment granules.

To test the rate of action of $HgCl_2$, spherical to oval blastulæ were placed in a concentration of 1/2,500,000. The embryos transferred to sea water after 5 minutes exposure were able to recover with only slight evidences of retardation in 24 hours. A 10-minute exposure visibly retarded development. More gastrula and triangular stages were found, while the control showed medium plutei. After 15-minute exposure, fewer individuals were able to gastrulate. These decreased in numbers following exposures of 20 to 45 minutes. During this interval more of the embryos were killed and movement became progressively slower. An exposure of 55 minutes killed most of the embryos, though a few survivors showed attempts to gastrulate.

This metallic salt does not appear to affect the gastrulation process independent of other developmental processes. As long as any survived they still attempted to gastrulate. Development went no further, however, during the period of observation. The most pronounced toxic effect on the majority of the embryos occurred within the first 15 minutes exposure.

$ZnCl_2$.—This metallic salt is slightly less toxic than $CuCl_2$. Gastrulation was inhibited in most individuals when the concentration was about 1/200,000. The most pronounced inhibitory effects appeared in concentrations up to 1/400,000. Persistent membranes appeared in the higher concentrations, and embryos failed to escape. Also an increasing number of embryos were killed in concentrations of 1/120,000 and higher. In these concentrations only an occasional attempt at gastrulation was seen; while in a 1/100,000 concentration, no attempt at gastrulation was seen and many were dead. On return to fresh sea water, the surviving individuals formed large globular structures without gut or skeleton. The ectoderm was often irregular and lumpy in appearance and movement was lethargic.

To test the rate of action of $ZnCl_2$ samples were transferred to fresh sea water from a 1/10,000 dilution every five minutes following

their introduction at 6:50 P.M. and were examined the following morning at 8–9 A.M. The control at this time showed young plutei. A 5-minute exposure gave blastulæ to late gastrulæ. Membranes persisted and some of the embryos failed to escape completely. The latter had not gastrulated. A 15-minute exposure gave blastulæ to young gastrulæ. Many had died and disintegrated or failed to escape from their membranes. The toxic effect of this metallic chloride is manifested very quickly after exposure. The most pronounced effects occurred within the first 10–15 minutes. After this the noticeable changes occurred very gradually. Thus even after one-half hour of exposure, gastrulation of many of the surviving embryos did occur during the experimental period. Movement, however, was very lethargic. After 1-hour exposure no normal gastrulæ were found, although some had attempted it.

$ZnSO_4$.—A stock solution of 0.1 per cent was used. As seen in Table IV, a concentration of 1/500,000 caused conspicuous retardation. In a 1/250,000 concentration, some individuals failed to gastrulate and those which did gastrulate were still in the young gastrula stage after 15 hours exposure. In a concentration of 1/175,000 most failed to gastrulate and some died. The hardened membranes failed to dissolve. The few individuals which attempted to gastrulate showed very broad invaginating regions. This effect was also observed in the $ZnCl_2$ study. In a 1/100,000 concentration only an occasional individual showed an attempt to gastrulate.

Zn Acetate.—This salt gave results quite comparable to the other zinc salts used (see Table IV). It caused the membranes to persist and the initial gastrulæ in the higher concentrations showed broad blastopores. The blastocœl was packed with cells. Gastrulation seemed to be inhibited at concentrations of 1/100,000 and higher.

V

It is well known that cyclic changes in the distribution of marine invertebrates are often accompanied by changes in the chemical composition of the sea water. Reproduction, embryonic development and growth are dependent on the presence of various constituents but the relative proportions of the various necessary elements may vary without any essential detrimental results. The absence of a necessary element or its presence in a non-utilizable form naturally disturbs development while the presence of too much of the element (experimental study) will also bring about developmental changes. The latter probably does not play as important a part in marine life as does the lack of the necessary amount of the element, but nevertheless

the study furnishes information on the specific effect of the element upon development.

The chlorides of some heavy metals, especially $NiCl_2$, whose effects on gastrulation and subsequent differentiation in the sea urchin, *Arbacia punctulata*, have been described in this study, may be added to the growing list of physical and chemical agents which may provoke exogastrulation. As is well known from studies by others, some metals are more toxic than others (Table IV) and the more toxic ones exert their toxic effect on gastrulation very quickly. The relative toxicity of the metallic salts seems to be as follows: $HgCl_2 > CuCl_2 > ZnCl_2$, $ZnSO_4$, $Zn(C_2H_3O_2)_2 > PbCl_2 > Al_2Cl_6$, $CdCl_2$, $NiCl_2 > FeCl_2$. In the case of most of the salts employed, the initial effect is upon growth and differentiation. Retardation and inhibition become increasingly more conspicuous in progressively higher concentrations, while the gastrulation process is the last to be effected. Finally it has been found that certain of the metals, especially $NiCl_2$, give a larger number of exogastrulæ at certain concentrations than in some of the previous studies where other types of environmental modifications were made, but they are not as effective in provoking exogastrulation as $LiCl_2$ (Runnström). It would appear, therefore, that such different physiological processes as gastrulation, differentiation and growth have different thresholds of inhibition for the same toxic substance.

Information is given on the concentrations which inhibited gastrulation. Providing the exposure has not been long enough to injure the embryo fatally, gastrulation and even further development will take place in fresh sea water. The rate at which some recovery takes place depends upon the concentration employed and the length of exposure. No cases of fused embryos were found.

As seen in previous studies (*cf.* Runnström, Lindahl), differentiation of the ectoderm and even of mesoderm, within limits, may take place independently of gastrulation or of the formation of the gut tube. Of the three germ layers, ectoderm is the most resistant to injury. In general the types of inhibitory or retardational effects are similar to those produced by other environmental modifications and by other workers. These include the behavior of the skeleton forming mesenchyme, the development of body form and size, the relative proportions of the potential ectoderm and endoderm, the inhibition of the gastrulation process, etc. The various metals give quite similar results but at different concentrations.

Information is also given on the rate of action of a lethal solution of each metallic chloride tested. The more toxic the metal, the

quicker are inhibitory effects shown even in very dilute concentrations. In higher concentrations of certain heavy metals like Zn, Cu and Pb, the fertilization membrane does not disappear. In such cases, and even where partial escape has occurred, the embryos do not differentiate. This lack of digestion of the membrane may be due to enzyme-poisoning which is so characteristic of some of the heavy metal salts (Ishida, 1936).

Although the differentiation of organs and tissues in both gastrulated embryos and in those where exogastrulation has occurred has been of interest to numerous workers, the effect of environmental modifications on the relative proportions in the amount of ectoderm and endoderm has attracted much study (Runnström, Lindahl, Lindahl and Stordal, and others). By appropriate stimulation of the egg the embryo can be animalized or vegetalized and the differentiation followed. Furthermore, this phenomenon can be inhibited by various means. For example, Li salts will vegetalize the embryo while SO_4 deficiency will animalize (ectodermize) the embryo.

Chlorides of certain heavy metals, acting on the blastula stage, likewise produce ectodermal embryos which lack gut or any endodermal material as far as can be seen. All transitions between this and typical gastrulation occur. The ectodermal embryos differentiate skeletal spicules or rods, apical plate and sometimes stomodæum. If treatment has not been too severe, the exogastrulæ likewise differentiate these structures. Arms never develop but oral and anal flaps may appear and the body tends towards typical shape which, however, is modified by the abnormal distribution of skeletal material. In extreme cases only large globular, ectodermal embryos are found in which the skeleton does not go beyond the spicule stage and neither stomodæum or apical plate differentiates.

Interpreted in the light of Runnström's hypothesis, the heavy metals employed may effect in a differential manner the ectodermal and endodermal gradients which he believes to exist in the fertilized egg (ectodermization and endodermization of the embryo, Lindahl, 1936). It has been shown previously that these same gradients may be present at the blastula stage and hence the limits of the endoderm and ectoderm are not definitely established even at the oval blastula stage which immediately precedes gastrulation. It is doubtful if the limits are established even at gastrulation. Lindahl (1936) considers that the two gradients have different metabolic rates and also that hydrocarbon metabolism dominates the animal pole while protein metabolism dominates the vegetal pole. The heavy metals used in this study may therefore exert their toxic action upon these metabolic

processes or produce substances which give the same effect which would account for some of the various developmental abnormalities and inhibitory effects described above.

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THE CYCLE OF ORGANIC PHOSPHORUS IN THE GULF OF MAINE

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It is generally recognized that the fertility of the sea depends upon a cycle in which carbon, nitrogen, phosphorus and other substances are assimilated under the influence of photosynthetic processes in surface waters and are set free again by processes of digestion or decay. The total organic productivity of a region is limited to the rate at which this cycle is completed. While it is apparent that in many localities the principal limiting factor is the rate of restoration of the inorganic products of decay (NO_3, PO_4) to the surface or photosynthetic zone, little is known concerning the exact locus within the sea at which decomposition actually sets these substances free, or of the rate at which the cycle as a whole or in part is completed.

The concentration of the ultimate products of decomposition, such as NO_3 and PO_4, tell us little about these points since they are stable substances capable of accumulating over a long period of time to high concentrations, and of being transported far from their place of origin. The actual site of decomposition is better indicated by the presence of intermediate products of decay such as ammonia, nitrite, and organic compounds of nitrogen and phosphorus.

The present paper is an account of the distribution of phosphorus throughout the year at a standard station in the Gulf of Maine. At all depths the amount of phosphorus present has been measured in three forms: (1) inorganic phosphate (PO_4), (2) dissolved organic phosphorus, and (3) particulate organic phosphorus. The analysis of these data gives some indication of the magnitude of the synthetic and disintegrative processes undergone by phosphorus compounds in different parts of the water column at different times of year, and of the extent to which phosphorus is transported from one depth to another.

The station chosen for study was located in the deeper portion of the western basin of the Gulf of Maine, 30 miles northeast of Highland Light. The surface water in this region is apparently relatively free from strong non-tidal currents, being sufficiently far offshore to avoid the coastal drift which accompanies the freshening of the water along

[1] Contribution No. 127.

the margin of the Gulf. In this part of the Gulf, as the result of freedom from strong currents, there develops each summer maximal surface temperatures and maximal stability of the water column. In this location one also finds relatively deep water (230–270 meters) free from strong non-tidal currents. Preliminary measurements made by Dr. E. E. Watson with current-meter indicate maximal tidal velocities of 11 cm. per second at 40 cm. above the bottom, and 14 cm. per second at 177 cm. above the bottom. The oxygen content in the deep water below 200 meters is low, varying from 4 to 4.5 cc. per liter. The point chosen for study thus presents conditions in which there exists above the bottom a considerable column of water too poorly illuminated to permit of photosynthesis, which terminates at a depth of 40 to 50 meters (Clarke and Oster, 1934) and in which decomposition occurs in sufficient magnitude to maintain a low oxygen concentration, and one as well chosen as may be to avoid disturbances due to the non-tidal drift of the water.

TABLE I

Station positions and dates.

Atlantis Station Number	Date	Location	Depth of water
			meters
2440	May 18, 1935	42° 22′ N. 69° 35′ W	249
2468	August 20, 1935	42° 20′ N. 69° 32′ W	232
2493	November 8–9, 1935	42° 21.5′ N. 69° 32′ W	256
2495	February 26, 1936	42° 22′ N. 69° 33′ W	270
2558	May 14, 1936	42° 27′ N. 69° 31.5′ W	254

Stations were made on five occasions separated by intervals of three months, thus completing one yearly cycle. Their positions and dates are given in Table I. The approximate position is indicated by a circle in Fig. 1.

ANALYTICAL PROCEDURE

Inorganic Phosphate

Phosphate samples were collected in black bottles and analyzed for inorganic phosphate at room temperature on shipboard by the Deniges-Atkins method, except that the solution of stannous chloride used contained 0.1 gram $SnCl_2.2H_2O$ in 10 ml. of 1 to 10 hydrochloric acid. Salt effect correction factor as measured was 1.35. Corrections for salt error and reagent blank were both applied.

Particulate Organic Phosphorus

Samples of water of about 300 ml. volume were filtered on shipboard as soon after collection as possible.

The procedure by which particulate organic phosphorus was determined is as follows. The particulate matter is filtered out by suction on a precipitate of barium sulfate on a 3G4 Jena sintered glass funnel with polished surface. The barium sulfate precipitate is

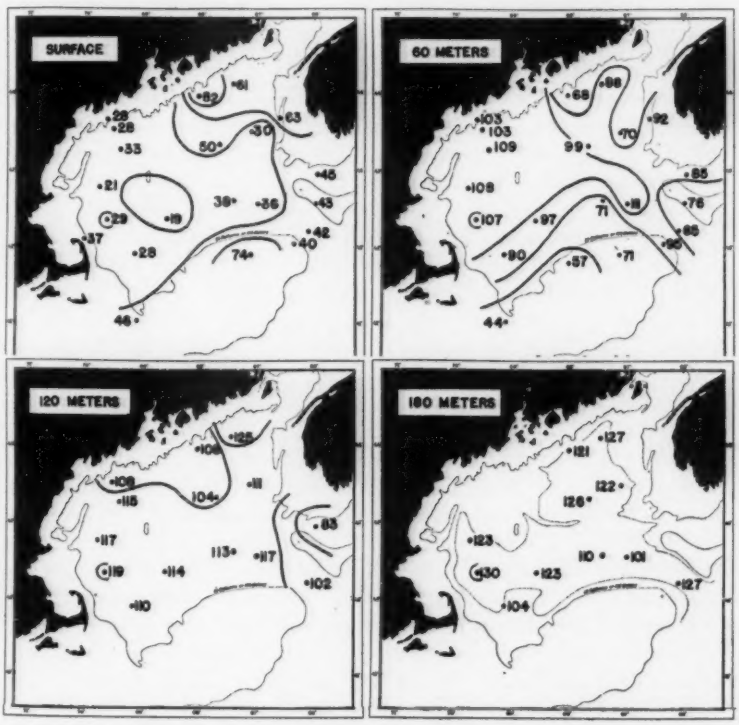

FIG. 1. The distribution of inorganic phosphate, PO_4, throughout the Gulf of Maine in May, 1934, at the surface and at the depths of 60, 120, 180 meters. The circle indicates the position of the stations at which the present investigations were made.

prepared by stirring 0.6 ml. of normal barium chloride into about 10 ml. of hot water containing excess sulfuric acid. It is poured over the funnel, the liquid sucked through, and filter and flask washed thoroughly with distilled water. The sea water sample is run through the

filter, and the volume of the filtrate measured. Plankton and barium sulfate are washed off the filter into a 125-ml. Erlenmeyer flask with a stream of distilled water. The sides and bottom of the filter are cleaned with a rubber policeman and all the precipitate transferred to the flask to insure removal of the plankton. The funnel is cleaned by reverse suction, treatment with sulfuric and chromic acids and thorough washing.

After the addition of 2 ml. of 38 per cent (by volume) sulfuric acid to the plankton, the flask is evaporated on the steam-bath to charring of the organic matter. The flask is ignited to fumes of sulfuric anhydride, and a drop of phosphate-free hydrogen peroxide (Cooper, 1934) (prepared by vacuum distillation of concentrated hydrogen peroxide) is added. A few seconds heating without loss of sulfuric anhydride fumes suffices to destroy the organic matter. The excess hydrogen peroxide is decomposed by heating the sample at 120° C. for one hour on an oil-bath. The cooled samples are diluted with ca. 50 ml. of distilled water and warmed on a steam-bath before filtering through a sintered glass funnel to remove the barium sulfate. The filtrate is diluted to 100 ml. in a glass-stoppered bottle. After the addition of 2 ml. of 2½ per cent ammonium molybdate each sample is shaken. Standards of similar phosphate content as potassium dihydrogen phosphate are made up with 2 ml. of 38 per cent sulfuric acid, 2 ml. ammonium molybdate and distilled water to 102 ml. and shaken. Two drops of stannous chloride solution containing 2.5 grams $SnCl_2.2H_2O$ in 100 ml. of 1 to 10 hydrochloric acid are added to each sample and standard with immediate shaking. After five minutes the samples are compared with the standards in a colorimeter with about 30 cm. depth of solution. Phosphate in the reagents for the development of the color (designated as Blank A) is determined by intercomparison of dilute standards. Phosphate introduced in the treatment of the unknowns (designated as Blank B) is determined by carrying through the procedure on a barium sulfate precipitate not treated with sea water. Blank A is added to the standards to give their true phosphate content. Both Blanks A and B are subtracted from the phosphate found in the unknowns. The net phosphorus in grams $\times 10^{-6}$ found in the unknowns multiplied by $\frac{1{,}000}{\text{ml. sample}}$ gives the plankton phosphorus in grams $\times 10^{-6}$ per liter. Errors in the analysis due to loss of phosphorus by volatilization or change in acidity by loss of sulfuric anhydride are shown to be negligible by carrying through blank analyses with known amounts of added phosphorus.

A procedure employing nitric acid as the oxidizing agent (Robinson

and Kemmerer, 1930), in which the nitric acid was evaporated off and the last traces removed by an evaporation with hydrochloric acid gave check results with the hydrogen peroxide procedure but a poor color match with the distilled water standards. It might be possible to use a procedure similar to that of von Brand (1935) for separation of the diatoms from sea water. No effort was made to differentiate between phosphorus and arsenic in the plankton phosphorus determinations. It was found, however, that the procedure of Zinzidze (1935) using bisulfate and sulfuric acid will bring about the complete reduction of at least 9×10^{-6} grams of arsenic to the limit detectable by the ceruliomolybdate method (2×10^{-7} grams) and make possible a distinction between phosphate and arsenic in plankton analyses.

Dissolved Organic Phosphorus

The phosphorus in solution in organic combination was obtained by subtracting the inorganic phosphorus from the total phosphorus in the filtrate from which the particulate matter had been separated. The following procedure for the determination of total phosphorus in a sea water sample was devised with a view to avoiding the interference of pentavalent arsenic with the ceruleo-molybdate phosphorus determination and to insure the complete destruction of organic matter. In the more rapid methods of analysis which have been used, in which sea water is treated directly with oxidizing agents, arsenic in the sample is oxidized and not subsequently reduced, while organic matter is not completely destroyed in solutions containing large amounts of chloride.

Fifty-milliliter samples of sea water are treated with 3 ml. of concentrated sulfuric acid (arsenic-free) in a 125-ml. Erlenmeyer flask. After evaporation on a steam-bath under a hood to carbonization of the organic matter, each sample is evaporated to the formation of fumes of sulfuric anhydride with swirling to avoid bumping. A drop of phosphorus-free 5 per cent hydrogen peroxide (prepared by vacuum distillation of 30 per cent hydrogen peroxide) is added. The flask is heated in the same manner without loss of sulfuric anhydride for half a minute more to make the solution colorless. If necessary, more hydrogen peroxide may be added. About 60 ml. of distilled water is added to the cooled sample, which is set on the steam-bath for complete solution of the precipitated salts. After transfer to a 500-ml. Erlenmeyer and addition of 8.5 ml. of concentrated ammonia water, the excess of ammonia is boiled off and the volume of solution reduced to about 30 ml. The sample is transferred to a small ground-glass-stoppered bottle and warmed on the steam-bath, open, with 2.47 ml. of 38 per cent (by volume) sulfuric acid. Four milliliters of 10 per cent

sodium sulfite (anhydrous, or hydrated salt in double the concentration) is added, and the stopper held in with a clip to prevent the escape of sulfur dioxide. Excess hydrogen peroxide is immediately destroyed, but eight hours heating on the steam-bath is necessary to reduce pentavalent arsenic to the trivalent form. The sample is transferred to a 500-ml. Erlenmeyer, boiled for five minutes to remove sulfur dioxide, cooled, and diluted to 100 ml. in a ground-glass-stoppered bottle. The residual acid in the sample is 2 ml. of 38 per cent sulfuric. After the addition of 2 ml. of 2.5 per cent ammonium molybdate the sample is shaken. Standards which will develop about the same color intensity as the unknowns are made up from a potassium dihydrogen phosphate solution with the addition of 2 ml. 38 per cent sulfuric acid, 2 ml. of 2.5 per cent ammonium molybdate solution, and distilled water to 102 ml. These are shaken. Each standard and sample is again immediately shaken after the addition of two drops of a solution containing 2.5 grams of $SnCl_2$ $0.2H_2O$ in 100 ml. of 1 to 10 hydrochloric acid. After four or five minutes for the development of the color the samples are compared with the standards in a colorimeter with about 30 cm. depth of solution.

Intercomparison of dilute standards in distilled water is made to estimate the phosphate in the reagents for producing the color (designated Blank A). A blank determination carried through with reagents alone gives the phosphate introduced by reagents in the treatment of the sample (designated Blank B). Blank A is added to the standards to give their true strength in calculating the phosphate in the unknowns. Both Blanks A and B are subtracted from the phosphate found in the unknown. The salt effect correction has been determined as 0.885 for unknowns by analysis of the same sea water with varying amounts of phosphate added. The slope of the graph of phosphate added against phosphate recovered is the salt effect correction. No variation in salt effect correction was found with salinity from 31.5 to 38.5. A similar procedure gave a salt effect correction of 0.81 in determining Blank B. Change of salt effect correction with temperature was not studied but extremes of temperature were avoided in making the analyses.

Since several samples of sea water gave identical analyses with or without the addition of as much as 165 mg. per cubic meter of arsenic (about eight times the amount found in sea water), arsenic was assumed to be reduced completely by the procedure used. Reduction of arsenic in solutions for determining Blank B was found to be much slower than in sea water samples and quantitative only for amounts of added arsenic equivalent to 50 mg. per cubic meter or less. The

difficulty was avoided by the use of reagents containing negligible amounts of arsenic. Schering-Kahlbaum "pro analysi" sulfuric acid was found suitable. The method can easily be adapted to use with a photometer although this was not attempted.

Table II, containing representative data from the analysis of samples of sea water, illustrates the procedure used in computing the dissolved organic phosphate, and gives some indication of the dependability of the methods. The total phosphorus in the samples obtained by combining the phosphorus in the filtrate with the particulate phosphorus retained by the filter (column IV) is compared with the total phosphorus in the water obtained by direct analysis without filtration (column V). It is evident that some small losses result from filtration, but these do not greatly exceed the normal variation of the Deniges-Atkins method. The values for the particulate phosphorus are small, but are consistent within themselves. The smaller values do not greatly exceed that of the blanks employed and in one set of data (for Station 2468, August 20, 1935) the values for the particulate phosphorus are in doubt owing to uncertainty in the value of the blank. The values for dissolved organic phosphorus in these samples are consistently positive and larger than the apparent errors of the method. During a large part of the year, as will be shown, much less soluble organic phosphorus is present in the water. Since this fraction is obtained by taking the difference of two large measures, each subject to considerable errors, it sometimes eventuated at such times that negative values were obtained for this fraction. The methods employed evidently do not give an exact measure of the distribution of the phosphorus fractions, but they do serve to indicate the general magnitudes of the quantities in which each occurs.

The meaning of the particulate and dissolved fractions of organic phosphorus depends upon the properties of the barium sulfate filter. When a suspension of diatoms, *Nitzschia closterium*, containing a known amount of phosphorus is filtered, the phosphorus may be recovered quantitatively from the filter. The refiltration of a filtrate leaves no detectable amount of phosphorus upon the filter. Evidently diatoms and microorganisms of similar size are completely retained in the particulate fraction. Dr. Charles E. Renn has kindly tested the filter for the retention of bacteria. After filtering sea water containing some 400 bacteria per milliliter the filtrate contained about one-sixth that amount. The particulate organic phosphorus fraction probably contains the phosphorus of all the larger phytoplankton and smaller zoöplankton and the greater portion of the bacteria and detritus as well. The dissolved organic phosphorus fraction may include a small

TABLE II

Specimen of data on phosphorus fractions in samples of sea water. Concentrations expressed as milligrams PO_4 per cubic meter.

Depth in meters	I Inorganic phosphorus	II Particulate phosphorus	III Phosphorus in filtrate	IV Total phosphorus II + III	V Total phosphorus. Direct analysis	VI Difference	VII Organic phosphorus III − I
1	45	10	64	74	62		
			70	80	86		
			68	78			
			67	77	71	+ 6	22
10	27	13	64	77	68		
			60	73	86		
			64	77			
			63	76	77	− 1	36
20	39	14	66	80	86		
			70	84	86		
			45	59			
			60	74	86	−12	21
60	110	6	116		130		
					116	− 1	6
				122	123		
100	110	4	126		142		
					142		
				130	142	−12	16
125	115	5	132		138		
					146		
				137	142	− 5	17
					160		
					156		
150	111	5	142	147	158	−11	31
200	123	5	152		154		
					162		
				157	158	− 1	29
225	108	8	148		162		
					158		
				156	160	− 4	40

portion of the bacterial flora, perhaps some minute nannoplankton, and detritus which has been reduced to the smallest dimensions in addition to organic compounds of phosphorus in colloidal form or in true solution. Renn (1937) has estimated that a bacterial population of 100,000 cells per milliliter would represent only 2.9 mg. PO_4 per cubic meter. It is evident that the much larger values of dissolved organic phosphorus obtained in our analyses can be due in only negligible part to the presence of bacteria. We believe, consequently, that our measurements represent chiefly the presence of phosphorus compounds in solution.

TABLE III

Inorganic phosphorus. Concentrations expressed in milligrams PO_4 per cubic meter. Depths not corrected for wire angle, which in no case would reduce by more than 4 per cent.

Depth in meters	May 12 1935	August 21 1935	November 8–9 1935	February 26 1936	May 14 1936
1	35	5	45	105	14
10	28	21	27	102	10
20	34	53	39	103	15
30	59	96	78	99	82
40	70	92	73	100	95
50	—	—	77	96	97
60	97	93	110	88	116
80	105	110	89	99	117
100	108	109	110	95	120
125	114	107	115	99	129
150	122	122	111	96	147
175	138	132	123	131	152
200	146	137	123	141	146
225	161	136	106	143	157
250	170	139		139	

The distribution of phosphorus in the three forms into which it has been separated is recorded in Tables III, IV, V and VI. Inorganic phosphate represents by far the greater quantity of phosphorus in the water amounting to 72 to 92 per cent of the total at different times. It is rather uniformly distributed at depths greater than 80–100 meters throughout the year, the concentrations increasing somewhat with depth. In the superficial layers, as has been frequently observed elsewhere, the inorganic phosphate becomes greatly reduced in quantity in the spring and is restored to concentrations characteristic of greater depths during the winter.

Particulate organic phosphorus, representing organisms and detritus, is in general the smallest of the three fractions, amounting to about 5 per cent of the total. It occurs in greatest quantity in the

upper layers—above 40 meters—corresponding to the observed distribution of phytoplankton (Gran and Braarud, 1935). The quantities in these layers are highest in spring; in midwinter the quantity in surface water is scarcely to be distinguished from that in deep water. The values obtained in August are subject to doubt. Below the photosynthetic zone the distribution of filterable organic phosphorus is on the whole very uniform, amounting to about 5 γ PO_4 per liter. The distribution and magnitude of the concentrations of filterable phosphorus agree well with that of particulate nitrogen observed by von Brand (1937) in these waters in the summer of 1936.

TABLE IV

Particulate organic phosphorus. Concentrations expressed in milligrams PO_4 per cubic meter. Depths not corrected for wire angle, which in no case would reduce by more than 4 per cent.

Depth in meters	May 18 1935	August 21 1935	November 8–9 1935	February 26 1936	May 14 1936
1	20	13	10	6	17
10	18	14	13	5	13
20	15	15	14	5	20
30	20	7	9	4	12
40	12	8	7	4	0
50	—	—	5	4	8
60	5	5	6	6	9
80	8	6	4	4	12
100	5	5	4	4	3
125	4	3	5	3	2
150	3	—	5	3	3
175	5	3	13	3	1
200	4	3	5	3	7
225	4	4	8	4	7
250	6			5	

In May dissolved organic phosphorus occurred in only minimal quantities in the water of all depths except near the surface. During the summer the concentration increases markedly until November, when over 20 per cent of the total phosphorus is in this form. In the early winter there is a rapid disappearance of this form of phosphorus, associated in time with the increase in concentration of inorganic phosphate in the upper waters. The appearance of organic phosphorus commences in May at the surface, and the concentrations appear to grow from the surface downward. Not until November are high concentrations observed near the bottom. These observations suggest that considerable decomposition is taking place throughout the water column and in particular in those depths where phyto- and zoöplankton

are known to exist in greatest numbers; and that at this station decomposition at the bottom may be relatively unimportant. The findings concerning organic phosphorus are somewhat similar to those of Kreps and Osadchik (1933), who made studies in Barents Sea. They found organic phosphorus to show a gradual increase from August to January reaching concentrations of some 40 mg. P_2O_5 per cubic meter and actually exceeding the inorganic phosphorus during the latter month. Their observations, which did not cover the earlier part of the year, showed the greatest concentrations in the deeper waters. As will be pointed out in a subsequent publication, the seasonal distribution of soluble organic phosphorus has some resemblance to

TABLE V

Dissolved organic phosphorus. Concentrations expressed in milligrams PO_4 per cubic meter. Depths not corrected for wire angle, which in no case would reduce by more than 4 per cent.

Depth in meters	May 18 1935	August 21 1935	November 8–9 1935	February 26 1936	May 14 1936
1	30	49	21	− 2	12
10	15	36	35	0	0
20	−1	50	29	22	35
30	−4	14	7	14	18
40	3	24	36	− 3	3
50	—	—	43	5	3
60	8	36	7	1	26
80	0	24	50	9	55
100	2	30	24	21	22
125	−3	29	20	21	22
150	2	6	38	22	9
175	−6	20	20	4	−11
200	−7	22	30	6	8
225	−2	17	43	− 4	10
250	8			−10	

that of ammonia, which may be considered to be a somewhat analogous stage in the nitrogen cycle.

The data recorded in Tables III, IV and V have been submitted to further analysis with a view to determining in so far as possible, just what alterations take place in the phosphorus cycle at various depths and at different times of year.

The column of water is considered to be virtually a closed system in which every exchange with the surroundings is exactly balanced by an equal and opposite exchange. By dividing the column into a number of segments lying at different depths, in which the quantity of phosphorus in the different forms is recorded from time to time, and by

observing certain general biological and hydrographic principles, it becomes possible to estimate to what extent changes in the concentrations of each form of phosphorus may be derived from processes taking place in situ, and to what extent vertical movements of phosphorus from one segment or layer to another must be postulated.

To obtain a workable body of data, Table VI has been drawn up recording the quantity of phosphorus present in each of the three

TABLE VI

Summary of distribution of phosphorus fractions.

	Depth in meters	May 18 1935	Aug. 20 1935	Nov. 8 1935	Feb. 26 1936	May 14 1936
Total P as grams PO_4 per sq. m.	0–240	28.7	31.0	32.9	29.9	34.4
Percentage of total phosphorus in each 60-meter layer	0–60	16.4	20.6	18.0	22.0	14.9
	60–120	23.7	23.2	25.0	23.0	28.0
	120–180	27.0	26.3	28.0	25.0	27.9
	180–240	32.9	29.9	29.0	30.0	29.2
	Total	100.0	100.0	100.0	100.0	100.0
Inorganic phosphorus as percentage of total	0–60	11.9	12.6	11.3	19.7	10.6
	60–120	22.1	16.8	18.7	19.4	20.8
	120–180	26.0	22.5	21.6	21.1	25.0
	180–240	32.0	25.6	21.2	28.7	26.5
	Total	92.0	77.5	72.8	88.9	82.9
Dissolved organic phosphorus as percentage of total	0–60	1.5	6.2	5.0	1.4	2.3
	60–120	0.4	5.4	5.4	2.7	6.0
	120–180	0.2	3.2	5.0	3.3	2.5
	180–240	0.0	3.7	6.4	0.6	1.7
	Total	2.1	18.5	21.8	8.0	12.5
Particulate organic phosphorus (organisms and detritus) as percentage of total	0–60	3.0	1.8	1.7	0.9	2.0
	60–120	1.2	1.0	0.9	0.9	1.2
	120–180	0.8	0.6	1.4	0.6	0.4
	180–240	0.9	0.6	1.4	0.7	1.0
	Total	5.9	4.0	5.4	3.1	4.6

forms for each of four layers each of 60 meters depth at each time of observation. The values are obtained by graphical integration and are expressed as percentages of the total phosphorus in a water column of 240 meters depth at each time of observation. In analyzing these data the following premises are held:

1. *The horizontal exchange due to the drift of water past the station may be neglected.* This premise is not justified on the ground that the

station is located in a region of minimal drift. There can be little doubt that water is constantly drifting past the station. The observed changes in salinity demonstrate this. The total phosphorus recorded varies ± 7 per cent from the mean value throughout the year. Since this variation shows no seasonal sequence, the total phosphorus being lowest in May, 1935 and maximal in May, 1936, there evidently is some variation in the character of the water occupying the station at different times. These differences are eliminated by expressing the phosphorus fractions as percentages of the total, a procedure which imposes artificially the character of a closed system upon the set of data. The justification of this procedure lies in the relatively small differences in total phosphorus observed from time to time, and in the fact that on the whole the horizontal distribution of phosphorus throughout the Gulf at any time, at least to judge by inorganic PO_4, is much more uniform than is the vertical distribution. The horizontal distribution of PO_4 at various depths throughout the Gulf as observed in May, 1934 are shown in Fig. 4 and illustrate this fact. The general character of the phosphorus cycle may be supposed to be similar in all parts of the basin.

2. *Phosphate present as zoöplankton and nekton and not sampled by the water bottle may be ignored.* A large number of vertical zoöplankton hauls made throughout the years 1933–34 in all parts of the Gulf yielded an average catch of 40 cc. dry plankton per square meter of surface. It may be estimated from analyses made on such material that this would contain about 0.4 per cent of the total phosphorus in the water from which it was strained. Since the particulate organic phosphorus amounts to about ten times this quantity, it may be seen that the neglect of this fraction does not introduce a significant error.

3. *All synthesis of particulate or soluble organic phosphorus compounds from inorganic phosphate takes place in the upper layer.* This is justified by Clarke's measurements on the penetration of light into the Gulf of Maine and on determinations of the compensation point in photosynthesis by diatoms in bottle experiments at different depths (Clarke and Oster, 1934).

4. *All downward movement of phosphorus is due to the sinking of organisms (particulate organic phosphorus).* This is the only fraction affected by gravity. It is also the only fraction displaying a well-marked concentration gradient decreasing downward—a condition essential for downward dispersal by eddy conductivity.

5. *All upward movement of phosphorus is due to the transport of inorganic PO_4 by eddy conductivity.* The gradient of concentration of inorganic PO_4 increases downward and is well marked except in mid-

winter. The soluble organic phosphorus never develops a strong gradient in this direction. Since it is present in much smaller concentrations than is the inorganic phosphate, it may safely be ignored in considering vertical transport by eddy conductivity.

6. *All observed transformations in any layer are attributed to processes occurring in that layer, except so far as vertical transport must be postulated to account for the transformation.* This premise is introduced since without it a unique solution cannot be obtained. It implies that all values arrived at for vertical exchange are minimal.

7. *The portion of the exchange in which the cycle runs to completion is necessarily ignored. All values for the magnitude of the exchange are consequently minimal.*

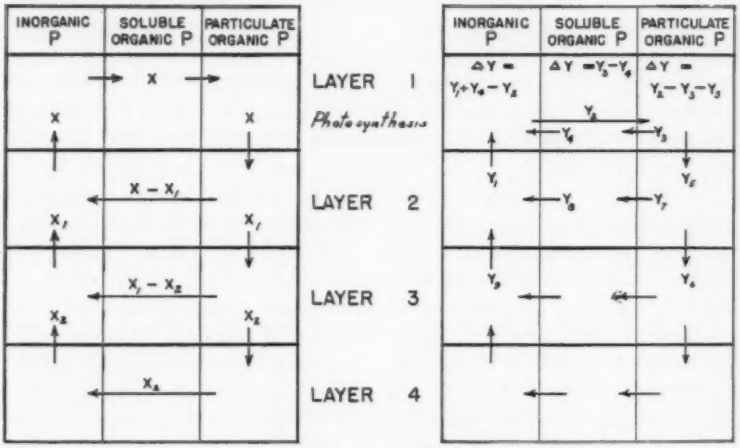

FIG. 2. See text.

A consideration of this limitation may serve to make clear the general basis of the analysis. If we start with the system in a steady state and if, during the period between two sets of observations the cycle has proceeded without change in the relative velocity of the processes in any part, the distribution of the fractions of phosphate in all parts of the system will be the same at the end as it was in the beginning. This does not mean that no exchanges of phosphate between different parts of the system have taken place, rather that all exchanges are exactly compensated. While the system is in a steady state an unobservable quantity of phosphate is undergoing transformation from each stage in the cycle to the next stage. If the system is disturbed, as through seasonal changes in the physical conditions, then

transformations of one sort may proceed more rapidly than those of another with the result that differences in the distribution of phosphorus are observed, and from these differences the magnitude and nature of the processes *which have caused* the differences may be deduced. The observations tell us nothing, however, of the basal level of activity on which the differences are superposed.[2]

Figure 2 illustrates the principle of the method. At any time there will be a basal level of activity represented by the transformation of an unobservable quantity of phosphorus, x, through each stage in the cycle. This quantity will have been transported upward from layer 2 into layer 1 to be synthesized into particulate organic form. If the system is to remain unchanged, the equivalent of this material must have sunk back into the deeper layers and been decomposed, passing through the soluble organic form, to exactly replace that which was transported upward. Portions of x, designated as x_1, x_2, etc. may sink to deeper layers before undergoing transformations from organic to inorganic form. The general conditions are that the quantity of x entering and leaving any part of the system shall be equal, that x move upward as inorganic phosphate (Postulate 5), and downward as filterable organic phosphorus (Postulate 4), that it represent synthesis of filterable organic phosphorus only in the upper layer, and that it represent a transformation of organic into inorganic phosphorus in any layer.

If the system is disturbed between observations, then changes in the quantity of phosphorus, ΔY, in any form and part of the system may be observed. These changes may be accounted for only by additional exchanges between the various parts of the system. The problem is to determine the minimal additional exchanges of this sort, Y_1, Y_2, Y_3 etc., which will account for the change in each part of the system in accordance with the postulates laid down above. The general conditions are that ΔY, the change in any fraction in any layer shall equal the difference in Y_1, Y_2, Y_3 etc., the amounts of phosphorus entering or leaving that fraction and layer during the period between observations. Furthermore, ΔY must be accounted for so far as possible by exchanges taking place within the layer in question (Postulate 6).

An attempted analysis of the changes in phosphorus distribution is presented in Tables VII, VIII and IX.

February to May (Table VII) represents the period in which the

[2] The method is analogous to the integration of a differential equation, the unobserved basal activity corresponding to the constant of integration. It is only in proportion as the system undergoes great seasonal fluctuation that the partial effects observed approach the total exchanges taking place. The method is applicable consequently particularly to studies made in high latitudes.

great spring flowering of phytoplankton occurs. During this period an amount of phosphorus equivalent to 9.1 per cent of the total disappears from the inorganic phosphate of the upper layer. Of this only 2.0 per cent can be accounted for as an increase in particulate and soluble organic phosphorus remaining in that layer. Seven and one-tenth per cent must have sunk to the deeper layers following its synthesis into organic matter. Only one-eighth of the phosphorus absorbed in

TABLE VII

Balance sheet of phosphorus exchanges February 26 to May 14, 1936. Numbers represent the change in the phosphorus fractions as percentages of total phosphorus in water column.

Depths		Inorganic phosphorus	Soluble organic phosphorus	Particulate organic phosphorus
0–60 meters	Photosynthesis	−9.1	→	+9.1
	Decomposition		+0.9 ←	−0.9
		0 ←	0	
	Exchange with layer below	0	0	−7.1
	Net change	−9.1	+0.9	+1.1
60–120 meters	Exchange with layer above	0	0	+7.1
	Decomposition		+4.7 ←	−4.7
		+1.4 ←	−1.4	
	Exchange with layer below	0	0	−2.1
	Net change	+1.4	+3.3	+0.3
120–180 meters	Exchange with layer above	0	0	+2.1
	Decomposition		+0.9 ←	−0.9
		+1.7 ←	−1.7	
	Exchange with layer below	+2.2	0	−1.4
	Net change	+3.9	−0.8	−0.2
180–240 meters	Exchange with layer above	−2.2	0	+1.4
	Decomposition	0	+1.1 ←	−1.1
	Net change	−2.2	+1.1	+0.3

photosynthesis has remained as particulate matter in the upper layer. This observation accords with the conclusion of Harvey (1934) that several times more vegetation is produced during the spring flowering of diatoms in the English Channel than is found there at the time of its maximum.

To account for the increasing concentrations of inorganic phosphorus in the deeper layers after allowing for the greatest possible decomposition in situ at least 2.1 per cent must sink past the 120-meter

level and 1.4 per cent past the 180-meter level. The phosphorus removed from inorganic form in the upper layer by photosynthesis is redistributed during the spring through considerable depths by the sinking of particulate matter. It is unnecessary to assume that any of the particulate matter sinks beyond the lower level before undergoing decomposition, though it is possible that this may be the case.

May to November (Table VIII) includes the greater part of the

TABLE VIII

Balance sheet of phosphorus exchanges May 18, 1935 to November 8, 1935. Numbers represent the change in the phosphorus fractions as percentages of total phosphorus in water column.

Depths		Inorganic phosphorus	Soluble organic phosphorus	Particulate organic phosphorus
0–60 meters	Photosynthesis	−19.2	→	+19.2
	Decomposition		+3.5 ←	−3.5
		0 ←	0	
	Exchange with layer below	+18.6	0	−17.0
	Net change	−0.6	+3.5	−1.3
60–120 meters	Exchange with layer above	−18.6	0	+17.0
	Decomposition		+5.0 ←	−5.0
		0 ←	0	
	Exchange with layer below	+15.2	0	−12.3
	Net change	−3.4	+5.0	−0.3
120–180 meters	Exchange with layer above	−15.2	0	+12.3
	Decomposition		+4.8 ←	−4.8
		0 ←	0	
	Exchange with layer below	+10.8	0	−6.9
	Net change	−4.4	+4.8	+0.6
180–240 meters	Exchange with layer above	−10.8	0	+6.9
	Decomposition		+6.4 ←	−6.4
		0 ←	0	
	Net change	−10.8	+6.4	+0.5

growing season. The important feature of this period is the appearance of large quantities of dissolved organic phosphorus at all depths. One-fifth of all the phosphorus in the water is in this form in November. This material can have been produced only by photosynthetic processes taking place in the upper layer. It must have been set free for the most part by decomposition of the particulate fraction in the layer in which it is observed (Postulate 5). In order to account for the

quantities of dissolved organic phosphorus observed, a large vertical movement of inorganic phosphorus upward through all depths must be postulated as well as an equivalent sinking of organisms to the sites at which the soluble organic phosphate appears. Over 17 per cent of the total phosphorus in the water must pass through the zone of photosynthesis in the course of the six summer months. Since the account is balanced without supposing any phosphorus to pass from the organic

TABLE IX

Balance sheet of phosphorus exchanges November 8, 1935 to February 26, 1936. Numbers represent percentages of total phosphorus in entire water column.

Depths		Inorganic phosphorus	Soluble organic phosphorus	Particulate organic phosphorus
0–60 meters	Photosynthesis	0	→	0
	Decomposition		+0.6 ←	−0.6
		+4.2 ←	−4.2	
	Exchange with layer below	+4.2	0	−0.2
	Net change	+8.4	−3.6	−0.8
60–120 meters	Exchange with layer above	−4.2	0	+0.2
	Decomposition		0 ←	0
		+2.7 ←	−2.7	
	Exchange with layer below	+2.2	0	−0.2
	Net change	+0.7	−2.7	0
120–180 meters	Exchange with layer above	−2.2	0	+0.2
	Decomposition		0 ←	0
		+1.7 ←	−1.7	
	Exchange with layer below	0	0	−1.0
	Net change	−0.5	−1.7	−0.8
180–240 meters	Exchange with layer above	0	0	+1.0
	Decomposition		+1.7 ←	−1.7
		+7.5 ←	−7.5	
	Net change	+7.5	−5.8	−0.7

back to the inorganic form, a process which must certainly be taking place, this figure may be far below that actually obtaining.

November to February (Table IX) is marked chiefly by the regeneration of inorganic phosphorus, which increases by 16 per cent of the total, and by the equalization of the concentration of this fraction throughout the water column. The table shows that this regeneration is made to a large extent at the expense of the soluble organic phos-

phorus, that the transformation takes place throughout the entire range of depths, though greatest near the bottom. The decomposition of organic phosphorus compounds in situ and the vertical transport of inorganic phosphate are about equally important in effecting the equalization of the concentration of the latter throughout the water column.

The Mechanism of Vertical Transport

The foregoing analysis indicates that very considerable exchanges of phosphorus take place between various depths of water. At the same time these exchanges appear to diminish in extent as the depth increases. Downward movement has been attributed to the sinking of particulate matter under the influence of gravity. There appears to be no difficulty in considering that in depths of a few hundred meters organized particles of the dimensions of diatoms would sink to the bottom before undergoing decomposition. If such were the case very large quantities of phosphorus would be withdrawn from the water during each growing season. This may be the case in shallow waters, but it does not appear to be happening in the western basin of the Gulf of Maine. If so the total phosphorus in the water should show a marked seasonal change. The situation is probably complicated by biological considerations. Harvey (1934) has presented evidence that the stock of phytoplankton is grazed down by zoöplankton during the summer. This conclusion suggests that the zoöplankton are important active agents in converting particulate organic phosphorus into its decomposition products. Since these animals, and particularly the copepods, make extensive diurnal vertical migrations, and since some time must elapse between the taking of food near the surface and its elimination as waste products, they provide an agency for a limited vertical transport of organic material. From this viewpoint the zoöplankton become an important agency in maintaining the fertility of the water for phytoplankton, since they hasten the conversion of bound nutrients into inorganic form and prevent these nutrients from becoming unavailable by the sinking of particulate matter to great depths or to the bottom.

The vertical transport of inorganic phosphate is simpler since it can be effected only by the mixing of the water. It is pertinent to inquire whether the conditions are such as to permit of the amounts of transport deduced during the various periods of observation. The amount of a constituent, Q, passing through unit horizontal surface in unit time depends upon the gradient of concentration of the constituent

dc/ds and the coefficient of eddy conductivity, A (Austausch coefficient).

$$Q = Adc/ds$$

The coefficient of eddy conductivity, A, represents the volume of water exchanged through each horizontal unit surface in unit time.

The gradient of phosphate concentration observed in February, May and November is shown in Fig. 3. Between May and November, when large vertical movements have been deduced, a well-marked gradient exists particularly in the upper layers, as is required for such movements. In February this gradient has disappeared, and the

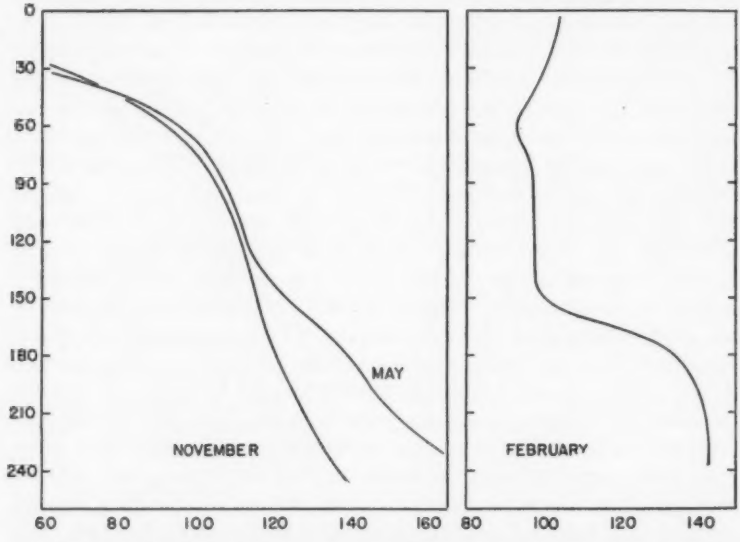

FIG. 3. Distribution of inorganic phosphate concentration with depth in November, 1935 and February and May, 1936 at standard station in the western basin of the Gulf of Maine. Depths in meters measured downward along the ordinate; concentrations in milligrams PO_4 per cubic meter along the abscissa.

concentration of phosphate is equal at all depths down to 150 meters. No amount of mixing can effect a change in its vertical distribution. Since this condition must exist during a considerable portion of the winter, it is not surprising that the vertical transports *deduced from our data* between November and May are smaller than those observed in the summer.

A knowledge of the coefficient of eddy conductivity, A, is the key to understanding the nutritive conditions in deep bodies of water.

Methods of estimating its value are so indirect that little is known of its magnitude under any circumstances. Although our data are admittedly very unprecise and yield only minimal values for the exchange, it is nevertheless of some interest to use it in estimating the coefficient required to account for the transport of phosphate in the Gulf of Maine. The gradient of phosphate concentration is sufficiently uniform throughout the period May–November to permit a single value to be taken at any level as representative of the entire period. This is not the case during the remainder of the year. We have

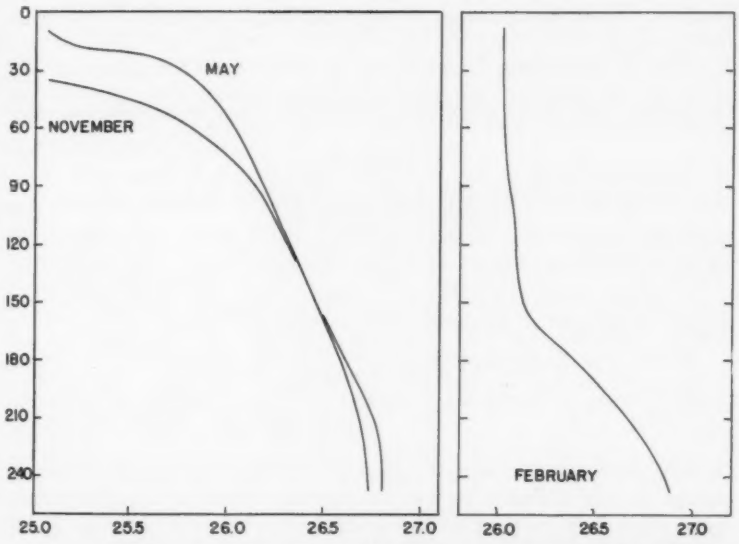

FIG. 4. Distribution of density, σ_t, with depth in November, 1935, and February and May, 1936, at standard station in the western basin of the Gulf of Maine. Depths in meters measured downward along the ordinate; density, σ_t, measured along the abscissa.

calculated the value of A for the boundary of each of the layers, using the data presented in Table VIII for estimating Q and the slopes of the curves in Fig. 3 for dc/ds. The result is shown in Table X. The values of A are minimal and are less reliable at the greater depths. The values obtained are not unreasonable. Seiwell considered A to equal 2 C.G.S. units in the thermocline of the tropical Atlantic, whereas values increasing to 50 C.G.S. units have been obtained in various waters (Seiwell, 1935).

The value of the coefficient of eddy conductivity depends upon the forces responsible for mixing and varies inversely with the stability, $\frac{d\sigma_t}{ds}$ of the water. If K represent the mixing forces,

$$K = A \times \frac{d\sigma_t}{ds}$$

Seiwell considers that $K = 4.73 \times 10^{-4}$ in the thermocline of the North Atlantic. Figure 7 shows the distribution of density, σ_t, with depth at the station in the Gulf of Maine in May, 1935 and in November. From these curves the representative values of $\frac{d\sigma_t}{ds}$ entered in Table X are taken. The values of K given by multiplying $\frac{d\sigma_t}{ds}$ by A are of the order obtained by Seiwell. It is concluded that the

TABLE X

Estimation of coefficient of eddy conductivity in Gulf of Maine, May to November, 1935.

Depths	Q		dc/dz	A	$d\sigma_t/dz$	$K = A \times \frac{d\sigma_t}{ds}$
meters	*per cent in 3 months*	$\frac{mg.}{cm.^3 \times sec.}$	$\frac{mg.}{ml.\ cm.}$	$\frac{ml.}{cm. \times sec.}$	$\frac{grams}{ml. \times cm.}$	
60	>18	$>3.5\times10^{-8}$	6.6×10^{-9}	> 5.2	1.0×10^{-4}	$>5.2\times10^{-4}$
120	>15	$>2.4\times10^{-8}$	2.0×10^{-9}	>12.0	0.5×10^{-4}	$>6.0\times10^{-4}$
180	>10	$>2.0\times10^{-8}$	3.0×10^{-9}	> 6.6	0.4×10^{-4}	$>2.6\times10^{-4}$

vertical transport of inorganic phosphate deduced from the seasonal change in the distribution of the various fractions of phosphorus compounds does not require unreasonable assumptions concerning the magnitude of the eddy conductivity.

SUMMARY

1. Methods are described for the determination of the phosphorus present in particulate form and of the total phosphorus in a sample of sea water.

2. The distribution of phosphorus present as inorganic phosphate, as dissolved organic compounds, and as particulate matter (detritus and microörganisms) has been determined at all depths throughout the year at a station in the western part of the Gulf of Maine.

3. In late winter over 90 per cent of the phosphorus is in inorganic form and three-quarters of the remainder is present as soluble organic compounds.

4. In the spring—February to May, inorganic phosphorus is converted to organic form by photosynthesis in the upper layer of water. Most of this fraction sinks to considerable depths before undergoing decomposition.

5. During the summer—May to November, large quantities of dissolved organic phosphorus appear at all depths, indicating a very considerable transport of inorganic phosphate from deep water to the surface and the sinking of an equivalent amount of phosphorus in particulate form to the depths in which organic compounds are liberated by decomposition. Decomposition appears to take place throughout the water column.

6. During the winter—November to February, the organic phosphorus compounds are converted to inorganic phosphate. This and vertical mixing of preformed phosphate are about equally important in bringing about the equalization of phosphate concentrations throughout the depth of water.

7. A method is described for analyzing quantitatively the factors producing a seasonal change in the distribution of a compound such as phosphorus. It is shown that the vertical transport of material within the water mass demanded by such an analysis may be accounted for reasonably by the hydrographic conditions obtaining.

8. Values of the coefficient of eddy conductivity at several depths are obtained.

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GROWTH AND VARIABILITY IN DAPHNIA PULEX

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INTRODUCTION

The aim of this study is to determine the number of pre-adult instars, growth, relative growth, and variability of individually reared female *Daphnia pulex* De Geer.

Numerous studies on growth of Cladocera have been made. Most of them are based on size-frequency distributions in natural populations. Some are based on experiments with individually reared animals. These studies have been carried on largely by Woltereck and his students, especially Rammner. Size-frequency distribution methods are inadequate for growth determinations as will be brought out more fully later. Previous studies carried out by means of individually reared animals deal with small numbers of organisms, a short part of the possible life span of an individual, or both. Recently Banta and his co-workers have carried out intensive studies on large numbers of individually reared *Daphnia longispina* which lived for long periods. In the present study a large number of animals has been observed for a relatively long time.

PROCEDURE

Essentially the same procedure was used as that employed by Anderson (1932) for *Daphnia magna*. Individual female *Daphnia pulex* of a single clone were isolated within eight hours after their release from their mothers and reared in manure-soil medium (Banta, 1921). Each individual was placed in a separate glass vial containing 20–25 cc. of the medium. Semi-weekly one-half of the total volume was replaced with fresh medium. The animals were kept at room temperature (15°–22° C.).

At the time of isolation and daily thereafter, each individual animal was placed in a watch glass together with a few drops of culture medium. Just enough saturated chloretone solution was added to bring about cessation of movement. Measurements as shown in Fig. 1 were made by means of an ocular micrometer. The animals were never in the chloretone solution for more than five minutes at any one time.

Immediately before the daily mensurations note was taken of cast carapaces and the number of young released. These were removed at the time of their discovery.

Results and Discussion

Longevity

Some 82 animals were observed in these experiments. Figure 2 is a survival curve. Fifty-one animals lived for twenty instars and of these, 3 continued to the twenty-fifth instar. Bourguillaut de Kerherve (1926) observed two *Daphnia magna* for nineteen instars. Each one released nineteen clutches of young and since not more than one clutch of young is released each instar and at least five instars precede the release of the first clutch of young, these two *Daphnia magna* lived

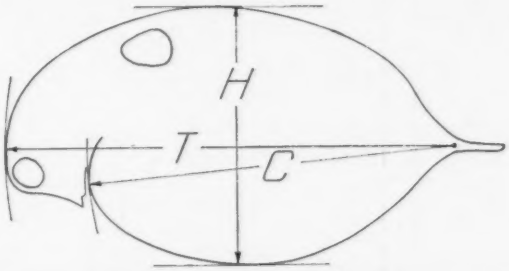

Fig. 1. Diagram showing method of making measurements. *T*, total length, longest dimension of animal exclusive of spine. *C*, carapace length, longest dimension of the carapace exclusive of spine. *H*, height, the shortest distance between two lines tangent to the carapace and parallel to the line of *T*. This measure of height is affected very little by the number of young in the brood chamber.

for twenty-four instars or more. He cites 4 other individuals which must have reached the twenty-second, the thirteenth, the tenth, and the sixth instar, respectively. Anderson (1932) observed some 30 *Daphnia magna* for fourteen instars, 32 for thirteen instars, and others for a smaller number of instars, all from the time of release from the mothers. Rammner (1928) observed one *Scapholeberis mucronata* for seventeen instars and another for nine. Rammner (1929) cites others that have been observed for shorter times. Ingle, Wood, and Banta (1937) have observed individually reared *Daphnia longispina* for over twenty-five instars.

Of the 82 animals observed in the present study, 71 were primiparous during the fifth instar and 9 during the sixth, while the remaining 2 died during the second and third instars. The number of

pre-adult instars is therefore variable in this species as well as in others (Anderson, 1932). The minimum number of pre-adult instars for *Daphnia pulex* is probably four.

Of the 71 animals primiparous during the fifth instar, 47 lived through the twentieth instar. The data from these 47, summarized in Table I, are the basis for the growth studies which follow.

Absolute Growth

Figure 3 is a group of growth curves in terms of total length, carapace length, and height. The curves are similar in shape. The

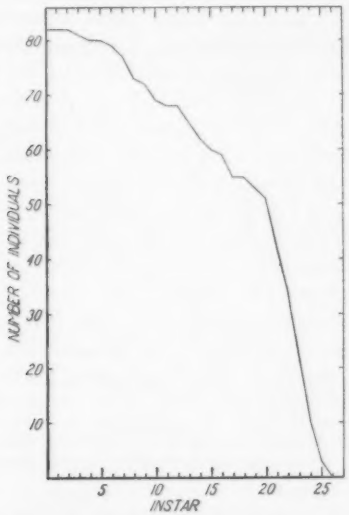

FIG. 2. Survival curve for the eighty-two animals observed in these experiments.

point of inflection in each curve comes immediately before the instar during which the animals are primiparous. Attempts have been made to fit the Robertson and Gompertz equations to the curves. In neither instance was the result judged satisfactory. The time unit used in this work is the instar. Adult instars have been considered as equivalent physiological time units (Anderson, 1933). At room temperature, each of the first three pre-adult instars lasts approximately one day, the fourth or last pre-adult instar one and a half days, the first adult instar about two days, and each subsequent instar becomes increasingly longer. The use of the instar as a time unit

may be the cause of unsatisfactory results in attempting to fit the equations to the present data.

When the logarithms of total length are plotted against time in instars, a curve is obtained which may be broken up into three distinct segments each of which approximates a straight line. The first segment takes in the first five instars, the second segment includes the next five, and the third segment embraces instars eleven to twenty.

Figure 4 shows the growth increments in terms of total length, carapace length, and height. The increments increase up to the fourth instar then gradually decrease until the eleventh instar, after which they remain much the same.

It is of interest to determine to what degree such characteristics of

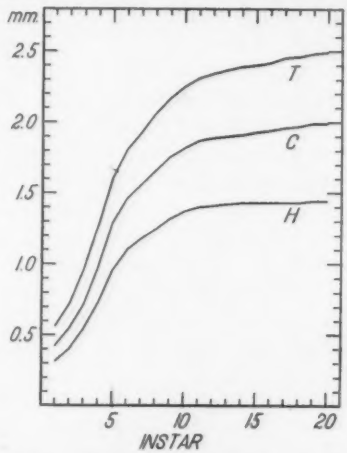

FIG. 3. Growth curves based on data from the 47 animals which were primiparous in the fifth instar and lived for twenty instars or more. *T*, total length; *C*, carapace length; *H*, height.

the growth process as initial size, final size, duration of growth, and initial velocity of growth are interdependent. As measures of these quantities, total length in the first instar (a), total length in the twentieth instar (A), the number of instars required to attain a length of approximately $0.8A$ (t), and the increment between the first and second instars (i), respectively, were employed. For these the following coefficients of correlation were obtained:

$$r_{aA} = 0.2309 \pm 0.0931$$
$$r_{ai} = -0.4481 \pm 0.0792$$
$$r_{Ai} = -0.1483 \pm 0.0963$$
$$r_{at} = 0.1641 \pm 0.0957$$
$$r_{At} = -0.3893 \pm 0.0835$$

It is apparent that only r_{ai} and r_{At} are significantly different from zero. There is thus an indication of an inverse relationship between initial size and initial velocity of growth, and between duration of growth and final size. On the other hand, there is no evident relationship between initial and final size, between initial size and duration of growth, or between initial velocity and final size.

Similar coefficients of correlation have been computed for *Daphnia longispina* by Wood and Banta (1936). These authors found that with unlimited food early growth tends to be inversely related to size at

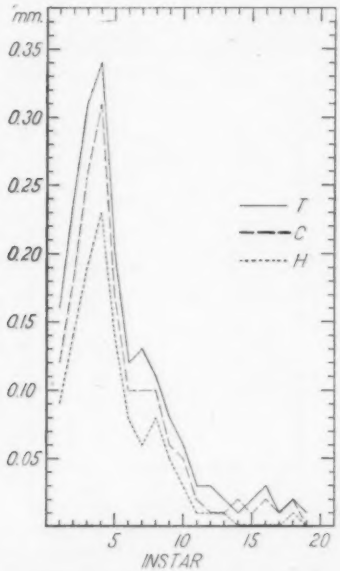

FIG. 4. Growth increment curves for the same animals as in Fig. 3. *T*, total length; *C*, carapace length; *H*, height.

the time of release, but that when the quantity of food is limited these factors vary independently. They found also that initially larger animals tend to become larger adults, which is contrary to the results obtained from our data.

A negative correlation between initial size and initial growth rate has been found to occur also in certain mammals. Thus it has been observed that in man (Hammett, 1918) and in the cat (Hall and Pierce, 1934) the percentage increase in body weight in the period immediately following birth is inversely proportional to birth weight.

Hammett (1918) interprets these results as indicating that differences in birth weight correspond to differences in physiological age at the time of birth, the physiologically younger individuals having the greater percentage growth rate.

In multiparous mammals the situation is often complicated by variation in litter size and attendant differences in food supply of the sucklings. Crozier and Enzmann (1935) showed that in inbred albino mice there is a hyperbolic relationship between birth weight and litter size, also that individuals in large litters grow more slowly at first than those in small litters, due to the decreased quantity of available milk per animal. After the suckling period, however, the growth of the smaller individuals is accelerated so that they eventually catch up with the others, and all attain the same adult weight regardless of birth weight.

In order to eliminate differences in nutrition, Kopeć (1932), working with a non-inbred stock of mice, reduced all litters to the same size. He found that birth weight varied inversely with litter size, and that individuals born in large litters exhibited a higher initial percentage growth rate than those born in small litters. When the data were seriated on the basis of birth weight without regard to litter size, however, there was found to be no correlation. Kopeć, who regards the different size groups in the second case as representing different genetic types, concluded that genetic differences in birth weight have no effect on subsequent growth, as do environmental differences. The validity of this argument seems open to question. It would be interesting in this connection to determine whether or not the same results occur with an inbred stock, also to investigate similarly the effects of brood size in Cladocera.

The occurrence of a significant negative correlation between duration of growth and final size in the present case appears to be in line with the results of McCay, Crowell, and Maynard (1935). These authors observed that in white rats, individuals whose growth period has been prolonged by partial starvation attain on adequate feeding a final size lower than that of controls given sufficient food throughout. Ingle, Wood, and Banta (1937), however, in a similar experiment on *Daphnia longispina*, found practically no difference in final size between the experimental animals and the controls. Also Merrell (1931) found that in rabbits there is no significant correlation between the time required to reach one-half the adult weight and the adult weight attained. On the other hand, Merrell's observation that initial growth rate is not associated with adult weight is in agreement with our results.

Reproduction

Figure 5 gives the average number of living young released during each instar. The number increases until the tenth instar followed by a gradual decrease. The period of increase corresponds to the time when the growth is falling most rapidly. The period of decrease corresponds to the time when growth has practically ceased.

The number of young released by a daphnid during any one instar has been used as an index of its condition (Anderson, 1933). The data available in the present study may serve as a test for this assumption. The young released in any instar develop from eggs produced during the instar before. Consequently, the coefficient of correlation must be computed from the growth increment with respect to total

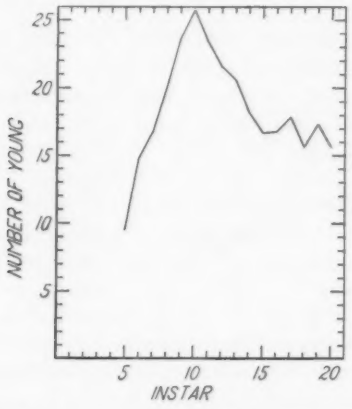

FIG. 5. Curve of the average number of living young released during each instar by the same animals as in Fig. 3.

length for instar five and the number of young released in instar six, etc. The coefficients for the fifth, sixth, and tenth instars were determined. These are .690 ± .025, .649 ± .028, and .645 ± .030, respectively. Hence one may conclude that the number of young produced may serve as a valid index of the condition of a daphnid.

Relative Growth

Relative growth studies show certain characteristics of this species. In Fig. 6 the logarithms of carapace length were plotted against those of total length, those of height against those of total length, and those of carapace length against those of height. The relation of carapace length to total length can be expressed satisfactorily on this *log log*

plot by three straight lines. The first of these can be drawn through the points for the pre-adult instars (1–4), the second through the points for the next eight instars (5–12) and the third through the last eight instars (13–20) represented. The relations between height and total length are similar to those between carapace length and total length. The relations between carapace length and height are different. Two lines may be used, one for the first ten instars and a second for the last ten (11–20). These linear relations can be expressed as

$$y = bx^{a},$$

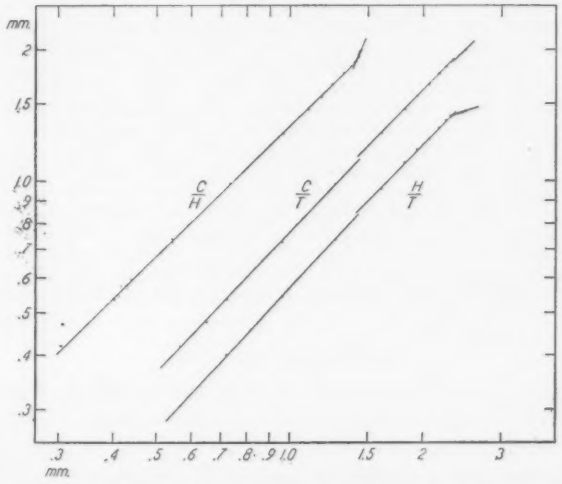

FIG. 6. Double logarithmic plots of the relations between carapace length and total length C/T, height and total length H/T, and carapace length and height C/H during each instar for the same animals as in Fig. 3.

where b is a constant—the initial growth index, a is the equilibrium constant, and x and y are values of two parts (Huxley and Teissier, 1936). The values of the constants are given in Table II. These values were calculated by the least squares method.

The relationships shown above are much the same as those secured for *Daphnia magna* by Anderson (1932). No very marked change in proportions occurs as may be seen in Fig. 7. The marked change in relative growth that occurs at the twelfth and thirteenth instars as indicated in Fig. 6 can hardly be recognized by inspecting Fig. 7 alone. The lines representing the thirteenth to the twentieth instars in Fig. 6 are very short since growth is very much retarded during this period.

TABLE I

Mean values of total length, carapace length, and height and their probable errors in millimeters for each instar. Also the standard deviation (σ) and the coefficient of variation (V) for total length.

Instar	Total length	Standard deviation	Coefficient of variation	Carapace length	Height
1	0.5665±0.0034	0.0347±0.0024	6.13±0.43	0.4194±0.0027	0.3056±0.0021
2	0.7214±0.0038	0.0389±0.0027	5.39±0.37	0.5361±0.0034	0.4015±0.0027
3	0.9609±0.0056	0.0568±0.0040	5.92±0.41	0.7244±0.0048	0.5433±0.0039
4	1.2653±0.0092	0.0934±0.0065	7.38±0.51	0.9848±0.0078	0.7346±0.0054
5	1.6079±0.0095	0.0964±0.0067	6.00±0.42	1.2850±0.0082	0.9597±0.0059
6	1.8134±0.0106	0.1081±0.0075	5.96±0.41	1.4572±0.0097	1.1015±0.0084
7	1.9332±0.0117	0.1196±0.0083	6.19±0.43	1.5591±0.0110	1.1778±0.0094
8	2.0571±0.0130	0.1316±0.0092	6.40±0.45	1.6615±0.0111	1.2445±0.0096
9	2.1673±0.0126	0.1275±0.0089	5.88±0.41	1.7587±0.0110	1.3213±0.0090
10	2.2466±0.0116	0.1174±0.0082	5.23±0.36	1.8200±0.0103	1.3738±0.0088
11	2.3061±0.0108	0.1095±0.0076	4.75±0.33	1.8653±0.0093	1.3976±0.0083
12	2.3401±0.0099	0.1007±0.0070	4.31±0.30	1.8891±0.0088	1.4131±0.0071
13	2.3705±0.0092	0.0939±0.0065	3.96±0.28	1.9034±0.0081	1.4227±0.0066
14	2.3853±0.0095	0.0962±0.0067	4.03±0.28	1.9135±0.0081	1.4262±0.0071
15	2.4032±0.0092	0.0935±0.0065	3.89±0.27	1.9326±0.0075	1.4274±0.0065
16	2.4223±0.0085	0.0866±0.0060	3.58±0.25	1.9433±0.0072	1.4274±0.0053
17	2.4461±0.0072	0.0736±0.0051	3.01±0.21	1.9594±0.0055	1.4340±0.0047
18	2.4604±0.0072	0.0730±0.0051	2.97±0.21	1.9689±0.0060	1.4340±0.0046
19	2.4835±0.0072	0.0730±0.0051	2.94±0.20	1.9862±0.0061	1.4435±0.0049
20	2.4890±0.0072	0.0731±0.0051	2.94±0.20	1.9928±0.0061	1.4429±0.0047

Variability in Body Size

Variability in body size and its fluctuations during growth have been studied in other forms by a number of investigators. The present data are particularly suitable for such a study, since it may safely be assumed that the animals are genetically homogeneous and that genetic differences are thus eliminated as a source of variation.

TABLE II

Values of the constants b and α for the data shown in Fig. 7.

Relation	Instars	b	α
Carapace length y.....	1–4	0.762	1.06
Total length x.....	5–12	0.790	1.03
	13–20	0.814	0.985
Height y.....	1–4	0.417	1.09
Total length x.....	5–12	0.530	1.03
	13–20	12.49	0.315
Carapace length y.....	1–10	1.333	0.99
Height x.....	11–20	0.848	2.34

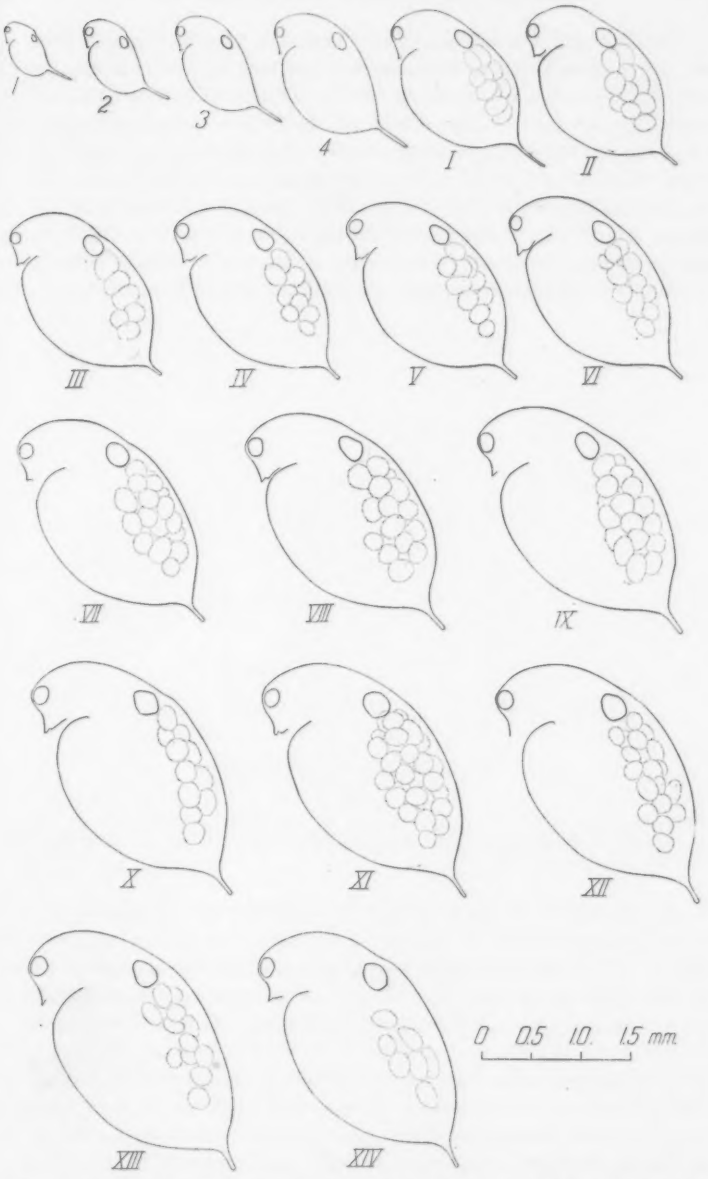

FIG. 7. Camera lucida outline drawings of a single animal for each of the first eighteen instars. Arabic numerals designate pre-adult instars; Roman numerals—adult instars.

Total length was employed as the best available measure of body size. Its use in this way is justified by the fact that there is apparently very little change in the shape of the body during growth (see the preceding section). The standard deviations and coefficients of variation for total length are given in Table I and are plotted against instar number in Fig. 8. It may be noted that both these quantities tend to increase at first, but that after the eighth instar they fall off rather steadily until the seventeenth instar, after which they remain approximately constant. Hence as the growth process reaches completion, body size becomes less variable both absolutely and relatively.

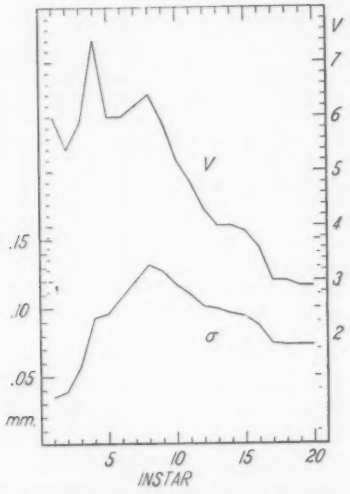

FIG. 8. Standard deviation and coefficient of variation of total length in relation to instar number.

A comparison of the curve for the coefficient of variation with the increment curve (Fig. 4) suggests that these two quantities are correlated. When the coefficient of variation is plotted against the logarithm of the increment (Fig. 9), the points tend to group themselves along a straight line, indicating that there is roughly a linear relationship between the relative variability and the logarithm of the growth rate. Whether or not any general significance can be attached to the precise character of the relationship as indicated here is open to question; however, there is no doubt that a distinct relationship exists.

These observations are essentially in agreement with those of previous investigators. A trend in the coefficient of variation similar

to that obtained here has been found to occur in the case of both stature and body weight in man by several workers, including Bowditch (1877), Thoma (1882), Porter (1894), Boas (1897), Boas and Wissler (1904), and others. A summary and discussion of the data of Bowditch, Boas, and Boas and Wissler is given by Thompson (1917, pp. 78–80). Essentially the same trend has been observed for body weight in the albino rat by Jackson (1913), King (1915), and Hanson and Heys (1927), in the Norway rat by King (1923), and in the cat by Hall and Pierce (1934). King (1918, 1919) has shown also that the trend persists in albino rats inbred for as many as twenty-five genera-

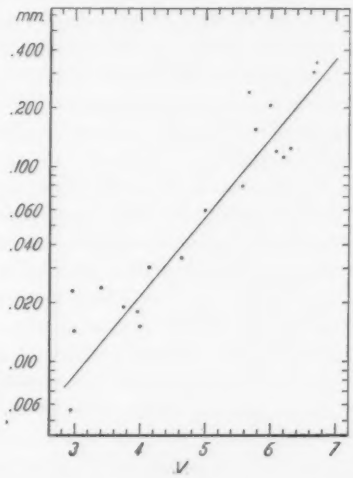

FIG. 9. Graph of the coefficient of variation of total length plotted against the logarithm of the increment. The values of the coefficient employed are averages of successive pairs in Table I.

tions, although the coefficient of variability in such rats is consistently lower than that in non-inbred animals of the same average body weight. Weymouth and McMillin (1930) have found that in the Pacific razor clam, *Siliqua patula*, relative variability in shell length, as measured by the ratio interdecile range: median, decreases steadily with increasing length.

Several of these investigators have remarked also the existence of a correlation between the coefficient of variation and the growth rate (*cf.* Thoma, 1882; Porter, 1894; Boas, 1897; Boas and Wissler, 1904; King, 1915, 1923). Jackson (1913), on the other hand, states that his data show no evident correlation between these quantities.

Thompson (1917) computed from the data of Boas the coefficients of variation for annual increments in human stature between the ages of five and eighteen, and found that these increased steadily with age. He attributes this to increasing differences in phase of growth among individuals of the group as growth proceeds. Analogous coefficients of variation computed for the present data fluctuate somewhat irregularly, but show on the whole a marked upward trend. In fact, from the thirteenth instar on, the values of the coefficients are considerably higher than 100 per cent. These values, however, are of doubtful significance, since the mean increments for these instars are small. When the value of the mean is close to zero, as Philiptschenko (1927) has pointed out, the coefficient of variation becomes unreliable as a measure of variability. As a matter of fact, one would expect that as the growth process comes to a close, the differences in phase of growth would tend to decrease (i.e. the increments for all individuals would ultimately become zero), and that variability in growth rate would correspondingly decrease.

The similarity of results obtained in the cases thus far investigated suggests that the relationships described above are of rather general occurrence, and that it may be worthwhile to seek a general explanation for them. In attempting to find such an explanation, it is necessary first of all to consider the sources of the observed variability, and the relative importance of each. There are in all four possible sources, namely errors in measurement, genetic differences, environmental differences, and the fundamental nature of the growth process itself.

It does not seem likely that the observed trend in variability can be accounted for to any significant degree on the basis of error in measurement. Assuming that the distribution of errors is Gaussian, we should expect that in measurements of length or weight, the absolute error, as indicated by the standard deviation, would remain constant or would, in some cases, increase with increasing magnitude of the object being measured. In the former case, the relative variability would decrease with growth; in the latter its behavior would vary, depending on the particular conditions. There is, however, no evident feature of such observational error which would account for a trend in the standard deviation such as that shown in Fig. 8, nor for the existence of a relationship between the coefficient of variability and the growth rate.

Some information concerning the rôle of genetic differences can be obtained from the data of King (1919) on the growth of inbred and non-inbred albino rats raised under identical laboratory conditions. Although the trend in the coefficient of variation is essentially the same

in both stocks, the degree of correlation between the coefficient and the growth rate, as indicated by the scatter of points on a graph, is much higher for the inbred rats than for the others. This may be interpreted to mean that variability due to genetic factors tends to be largely independent of the velocity of growth.

On the other hand, variability due to environmental differences would be expected to vary with the growth rate, for an individual in a state of rapid growth is relatively highly sensitive to the action of environmental agents, and a given fluctuation from the norm would produce a greater deviation in growth than it would in an individual with a low growth rate. This has been recognized by Plunkett (1932) in connection with developmental processes in general. He points out that as such a process asymptotically nears completion, the organisms tend to become more stable and less sensitive to the effects of environmental factors such as, for example, temperature. Hence the variability with respect to the particular character involved will decrease. However, he erroneously assumes that the converse is also true, namely that if one group of organisms is less variable in a certain respect than another group, it is therefore nearer to the completion of the underlying developmental process. This may or may not be generally true, but it clearly requires further demonstration.

It has generally been assumed that variability in growing organisms, aside from that introduced through errors in measurement, must be due to either genetic or environmental differences. According to a recent theory proposed by Rahn (1932), however, such variability arises at least in part from the physico-chemical nature of the growth process itself. The theory is briefly as follows. The division of a cell must be preceded by the doubling of all its genes. Let us consider a particular gene in each of a number of unicellular organisms which are identical with respect to genetic and environmental factors. If we assume that the doubling of this gene in the various cells conforms to the law of mass action, then it follows that it will not double simultaneously in all the cells, but will do so over a definite time interval. If the same is true of each of the remaining genes, there will be a variability in division time of the cells.

Rahn has developed a simple mathematical formulation of the theory which he has applied to data on the division rate of bacteria. For multicellular forms, he has succeeded in analyzing only the case of a hypothetical organism in which all the cells are alike and in which the percentage rate of cell division is constant. He has shown that such organisms will vary with respect to the time required for the completion of a given number of cell generations. Moreover, as the

number of generations increases, the frequency curves become flatter while the relative spread of variation decreases. In other words, there will be an increasing standard deviation and a decreasing coefficient of variation. If the theory is valid, it would follow that variability in cell number (and in body size insofar as it depends on cell number) in successive time intervals should behave in a similar fashion.

Obviously these conclusions are not directly applicable to growth in actual multicellular organisms for a number of reasons. First of all, such growth is generally characterized by a decreasing percentage growth rate; secondly, it involves increase in cell size, as well as in cell number; and thirdly, the average reproductive rates of different types of cells are not equal. Before the conformity of the theory with the observed results can be tested, the former must receive a much

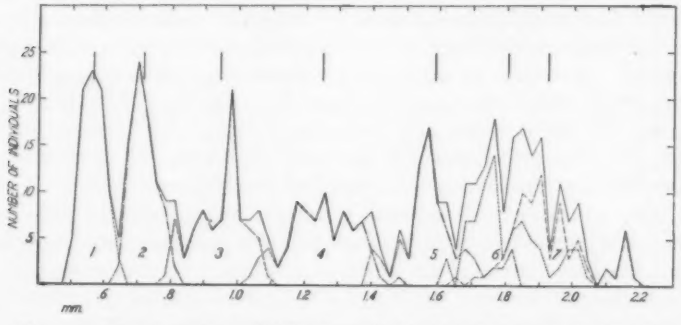

FIG. 10. Size frequency distributions during the first seven instars for all eighty-two animals observed. Broken lines designate the individual instars. The solid line is a composite curve for all instars. The vertical bars near the upper edge of the figure represent the mean total lengths for each of the first seven instars.

more elaborate mathematical formulation, and a theoretical analysis must be made in which these factors are taken into account.

It may therefore be tentatively concluded that the observed trend in variability in body size with growth and its relation to growth rate are explicable largely in terms of the action of environmental factors, and perhaps also in part in terms of Rahn's theory.

Size-frequency Distribution

Most studies of growth in Cladocera have been made by analysis of size-frequency distributions in natural populations. These have been of two types (see Woltereck, 1929). One consists of plotting the number of individuals in a size class against size. In the graph that results a number of size modes appear which are taken as repre-

sentative of the growth stages or instars. A second type consists of plotting the value for one dimension of an individual against another dimension of the same individual for each animal in a population. Usually the points fall into groups which are more or less distinct. Each group of points is taken as representative of a single instar.

No one so far as we are aware has attempted a comparison between the results secured by these methods and those secured by observations on individually reared animals. To make such a comparison, Figs. 10 and 11 were constructed. First the values for total length fre-

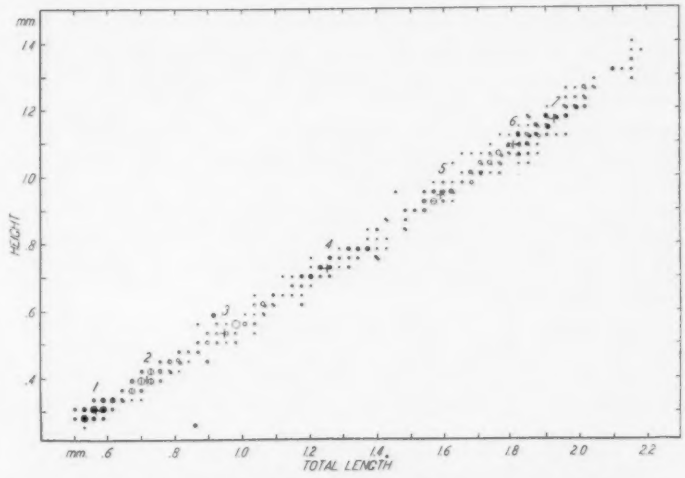

FIG. 11. Size frequency distributions in two dimensions during the first seven instars for all 82 animals observed. Each instar is represented by a different type of circle. The area of each circle is directly proportional to the number of animals of the particular dimensions represented by the circle. The crosses are the mean values for each of the first seven instars.

quency during the first instar for all 82 animals observed were plotted. Then those for the second instar and so on to the seventh instar. After that a composite curve for all the values was plotted. The separate instar curves are shown in broken lines and the composite curve in a solid line in Fig. 10. The vertical lines in the upper part of the figure represent the mean total lengths for each instar up to and including the seventh. It is obvious that each mode in the composite curve does not represent the mean total length for an instar. The first three distinct modes are near the mean values. No distinct mode is apparent for the fourth instar. The mean value for the fifth

instar falls near a mode but the means of the sixth and seventh instars fall midway between modes. Further, considerable overlapping occurs between the values for various instars. The greatest value for the first instar is larger than the smallest for the second. Similar are the cases for the second and third instars, the third and fourth, and the fourth and fifth. Some of the values for the fifth are greater than the lowest for the seventh instar. This size-frequency method may be of some use for the pre-adult instars, but certainly not for adult instars.

Figure 11 was constructed by plotting the values of height against that of total length for all 82 animals for the first instar, followed by those of the second instar, and so on through the seventh. The area of each circle is directly proportional to the number of individuals represented. Where values for two or more instars fall on a point separate circles are used for each instar. The mean height and the mean total length for each instar are represented by crosses. This method differs from that described by Woltereck (1929) in that each individual was represented by a point on the graph while in the present case if more than one individual is of the same size the circle at point on the graph is larger and its area proportional to the number of individuals represented.

In analyzing Fig. 11, it will be noted that a band of circles is secured but no distinct groups. The circles of the greatest area may be considered to represent the instars. For the first three instars, the circles of greatest area fall near the mean values for the instars. No conspicuously large circle is found to represent the fourth instar. The cross for the fifth instar falls near a large circle but the crosses for the sixth and seventh instars do not. In most plots of natural populations (Woltereck, 1929) the points scatter more widely, i.e., they form a wider band than is the case in Fig. 11. Here again it is obvious that size-frequency distribution does not lend itself to a satisfactory study of growth.

Studies on growth by means of individually reared animals are advantageous over the above methods in several respects. The individual life histories are fully known. Genetic constancy can be maintained since diploid parthenogenesis is the only means of reproduction. Selection of young from one clone insures genetic constancy except for mutations. The environment can be maintained fairly constant. By natural population analysis methods the individual histories are unknown, the genetic constancy cannot be controlled, and the environment is subject to considerable fluctuation.

Summary

Eighty-two individually reared female *Daphnia pulex* were observed from the time they were released from the brood chamber of their mothers until they died. Measurements of total length, carapace length, and height were made daily on each animal. The number of young released during each instar was recorded.

Seventy-one animals were primiparous during the fifth instar and 9 during the sixth. Consequently the number of pre-adult instars is variable, the minimum being four.

Data from the 47 animals which were primiparous during the fifth instar and which lived for twenty instars or more were used in constructing growth curves. Growth in the three dimensions studied is sigmoid. The point of inflection in all curves comes during the fourth instar, the last pre-adult.

The Robertson and the Gompertz equations do not fit the data satisfactorily. This may be due to the time unit employed, which in this case is the instar.

The growth increment is greatest during the fourth instar. The increments increase up to the fourth instar, then decrease gradually until the eleventh instar, after which they remain low and relatively constant.

A significant negative correlation exists between initial body size and initial growth rate, also between duration of growth and final body size. Other characteristics of the growth process investigated appear to vary independently.

The number of young released during the adult instars increases to a maximum at the tenth instar followed by a gradual decrease.

The number of young released during any adult instar is significantly correlated with the growth increment for the instar preceding the one during which the young are released.

Relative growth in the dimensions studied may be expressed satisfactorily by the equation

$$y = bx^{\alpha}.$$

No marked changes occur in the relations between total length and carapace length. Marked changes in the relations between carapace length and height and between total length and height occur at the thirteenth instar.

The standard deviation and coefficient of variation of total length tend to increase somewhat during the early instars, but after the eighth instar both decrease rather steadily.

Relative variability in body size, as measured by the coefficient of

variation of total length, is roughly directly proportional to the logarithm of the growth rate. The existence of this relationship is ascribed largely to the mode of action of environmental factors during growth. It may perhaps also be explicable in terms of Rahn's theory of the physico-chemical origin of variability in growth rate.

Size-frequency analyses of natural populations as methods of studying growth are shown to be inferior to the method using individually reared animals.

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SEASONAL PRODUCTION OF ZOÖPLANKTON OFF WOODS HOLE WITH SPECIAL REFERENCE TO CALANUS FINMARCHICUS[1]

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The purpose of the work described in this paper was the investigation of the distribution of the plankton in the waters around Woods Hole during the summer months and the study of the seasonal changes in the plankton at one locality throughout an entire year. Our primary object specifically was to ascertain whether the copepod, *Calanus finmarchicus*—a form used extensively for laboratory experiments—breeds in this region, and if so during which months and with what success. It was proposed also to include measurements of the physical and chemical factors in the environment, and the collection of nannoplankton and phytoplankton, in order that the sequence of biological events from season to season in this locality might be followed. Cause and effect relationships in the growth of the various plankton forms might thus be unraveled.

Since copepods play a prominent rôle in the economy of the sea, it is important to know what conditions promote their growth and what factors tend to reduce their numbers (Clarke, 1934). To this end a series of laboratory experiments was initiated in which the nutrition of copepods, particularly of *Calanus finmarchicus*, was investigated (Clarke and Gellis, 1935; Fuller and Clarke, 1936; and Fuller, 1937). The nearest point from which copepods of this species could be obtained in sufficient numbers was in the deeper water off Gay Head. The population of *Calanus* at this locality was found to persist throughout the summer, but we had no knowledge either of the subsequent fate of these copepods or of the time and conditions of their appearance here. In our studies of nutrition we had assumed that the *Calanus* found in this area were living in equilibrium with their environment and that the smaller organisms found in the same body of water were adequate both qualitatively and quantitatively for their food supply. If, however, these copepods had been produced in another locality and transported hither by currents, the possibility existed that the conditions at our point of observation were not suitable

[1] Contribution No. 112, Woods Hole Oceanographic Institution.

for the growth or even the maintenance of *Calanus* and that the copepods found here were destined shortly to die off. The proposed field observations were therefore especially desired and when worked out in conjunction with further laboratory experiments should eventually yield information on the factors controlling copepod production which would have a general application.

Let it be understood, however, that the work described in this paper was of the nature of a reconnaissance program. The locality chosen for the observations throughout the year was a point as far offshore as could be reached within a day's sail from Woods Hole, but it probably is not entirely removed from fluctuating land influences. However, evidence will be given below for believing that this station is typical of a considerable area.

Location of Stations and Hydrography

During the summer of 1935 the five stations in the waterways around Woods Hole shown in Fig. 1 were visited about twice a week. Buzzards Bay is a broad, shallow body of water with a temperature a few degrees higher and a salinity slightly lower than Vineyard Sound. A strong current flows in Vineyard Sound, and offshore water seems to be carried into the Sound much more readily than is the case with Buzzards Bay (Haight, 1936). Since access to the open sea from Woods Hole is most direct through the western entrance of Vineyard Sound, a line of stations was run in that direction to study the transition between inshore and offshore conditions and populations.

Station 3, which was selected for the continuation of the observations throughout the year, was visited once a month during the autumn and winter and at least twice a month from April to September. The authors are indebted to Dr. Renn, Mr. Iselin, Mr. Woodcock, and Mr. Butcher for assistance which made these trips possible. A rough sea is almost always encountered at the exposed location of Station 3, and during the extremely cold weather when spray froze on the deck and rigging, work was extremely difficult.

Station 3 is about eight miles from the nearest points of land on the north and east, and twenty-five miles from Block Island on the west. On the south the continental shelf water extends without obstruction. Since at Station H2 (see Fig. 1) the flood tide flows NNE and the ebb tide flows WSW, the main body of water debouching from Vineyard Sound and Buzzards Bay probably passes largely to the west of Station 3. However, the currents in this region are extremely complex and unfortunately the measurements of the U. S. Coast and Geodetic Survey (Haight, 1936) do not extend as far out

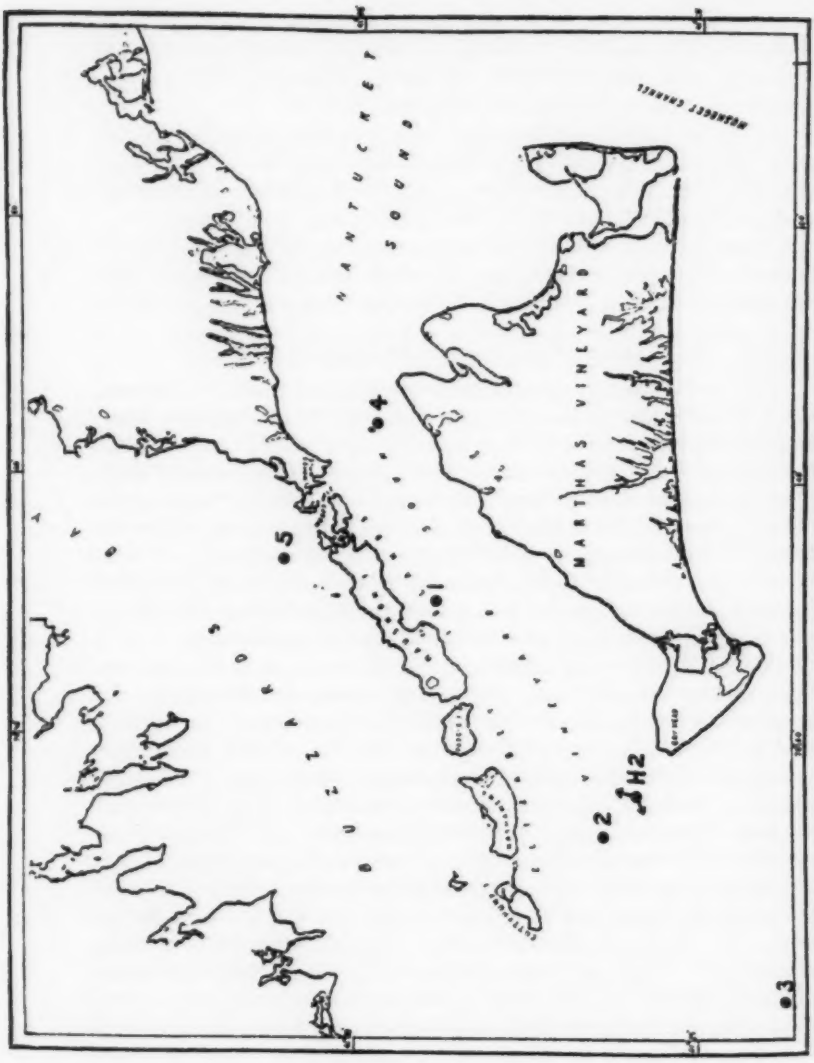

FIG. 1. Location of Stations 1–5 at which plankton hauls were made, and of Station H2 occupied by the U. S. Coast and Geodetic Survey. The small island of No Man's Land lies just off the map to the south of Gay Head. Buzzards Bay comes to an end just north of the limit of the map, whereas Nantucket Sound is open to the sea on both sides of Nantucket Island.

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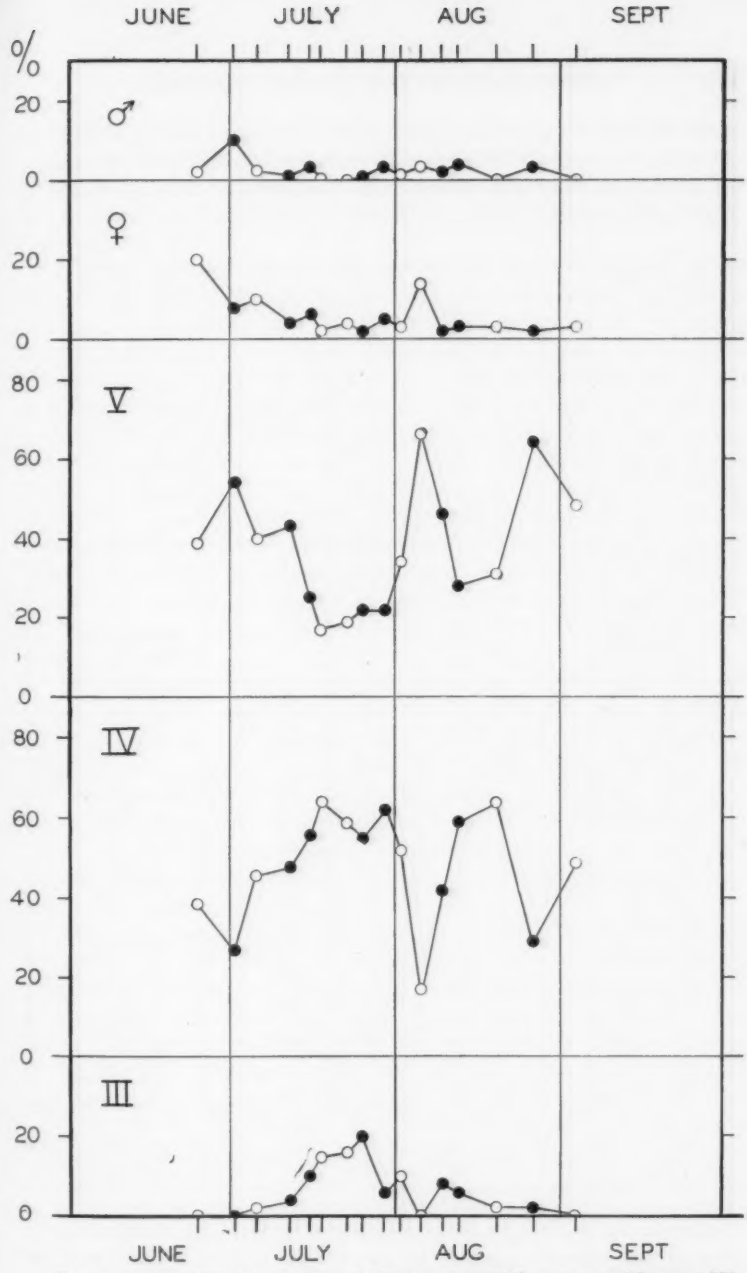

FIG. 2. Percentage distribution of *Calanus finmarchicus* copepodid stages III, IV, and V and adult ♀ and ♂ taken with the scrim net at Station 3 during the summer of 1935. The open circles represent catches made on an incoming tide (E. 1 hour–W. 1 hour) and the solid circles represent catches made on an outgoing tide (W. 1 hour–E. 1 hour).

as our station. Our plankton catches during the summer of 1935 at the stations nearer shore showed a considerable difference depending upon whether they were made on an incoming or an outgoing tide. There is some evidence that the effect of the tide was felt as far out as

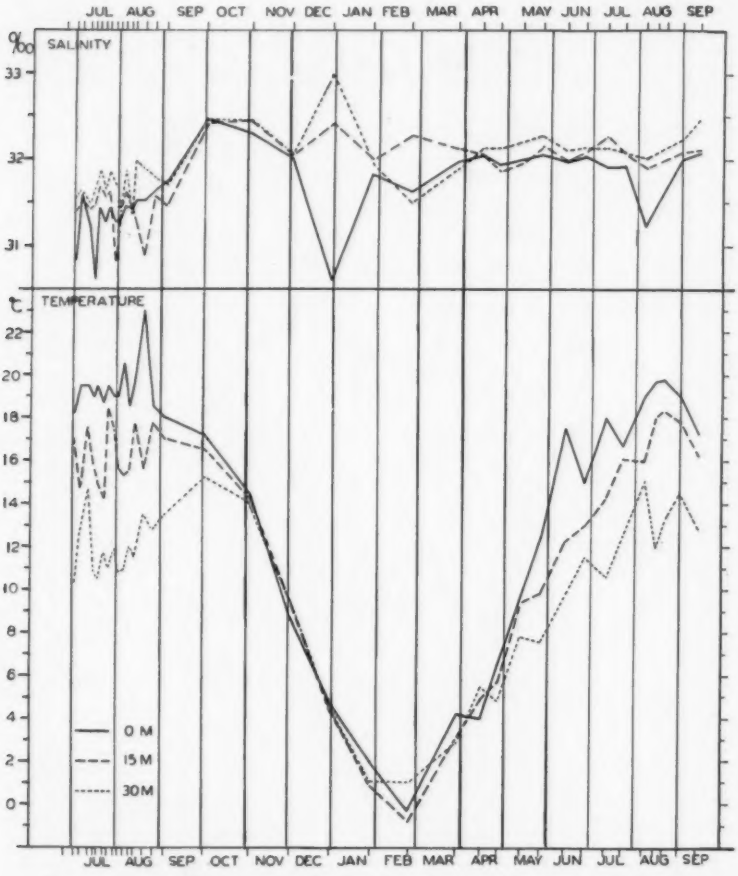

FIG. 3. The salinity and temperature at depths of 0, 15, and 30 meters at Station 3 during 1935–6. The vertical markers along the abscissa indicate the dates on which observations were made.

Station 2, but at Station 3 no consistent difference due to the phase of the tide was observed, as may be seen for the *Calanus* population from Fig. 2. Transparency measurements similarly indicated the presence

of typical offshore water at this station (Clarke, 1938). However, for the sake of uniformity, our observations throughout the year were made as far as possible only during the periods when the "east," or flood tide, had flowed for at least one hour, and before the "west" tide had flowed for more than one hour.

These tidal oscillations are superimposed upon the much larger but slower coastal current which flows in a southwesterly direction over the continental shelf all along this coast. Water is moving continuously from Nantucket Shoals on toward New York. It is presumably from the inner edge of this current that the water at our station is derived. Yet there must be a certain admixture of inshore water, since the salinity value at Station 3 (see Fig. 3) was slightly lower at all seasons of the year than that characterizing the water farther offshore[2] and was slightly higher than that found within Buzzards Bay and Vineyard Sound, according to determinations which we carried out during the summer of 1935. The prevailing wind, which is from the southwest, would tend to blow offshore water in toward our station, but gales from this direction or from other points of the compass probably disturb the normal hydrographic situation rather profoundly.

The fluctuations in the salinity at Station 3 from July, 1935 to September, 1936, were for the most part confined between 31.5 and 32.5 ‰ and generally uniform at the depths of 0, 15, and 30 meters (Fig. 3). On several occasions, however, a noticeable freshening of the surface layer was observed, and on December 29, 1935, an unusually great difference in salinity existed between the surface and the bottom. Since a pronounced transparency anomaly occurred on the same day (Clarke, 1938), we may assume that an abnormal interchange of water masses took place on this occasion.

The temperature change at Station 3 during the year was extremely great for a marine environment. The maximum value, observed in August, was over 23° C. higher than the minimum value, in February (*cf.* also Allee, 1919). During the summer months the water was highly stratified, for a difference of 8° or 10° was usually found between the surface and the bottom. This would seem to indicate that no great stirring was produced by wind or tide in these months, but it is conceivable that the temperature difference could be brought about by some persistent differential water movement. In September the surface water began to cool off rapidly and to mix more effectively with the bottom water, thus raising the temperature of the latter. By the first of November the temperature had become

[2] At Station "Martha's Vineyard I." See H. B. Bigelow and M. Sears (1935).

uniform from top to bottom. This condition persisted throughout the autumn and winter indicating strong stirring action during this period. In the spring months warming took place more rapidly in the upper layers than in the deeper with the result that a progressively greater difference in temperature was found between the surface and bottom until by June or July a pronounced stratification was re-established.

Methods

During the summer of 1935 10-minute horizontal hauls for zoöplankton were made using the scrim closing nets described by Clarke (1933). At Stations 1, 4, and 5 hauls were made at depths of 2 meters below the surface and 2 meters above the bottom; at Stations 2 and 3 an additional haul was made at a depth mid-way between the other two. Water samples were taken and temperatures determined at each of these depths. A fraction of each water sample was turned over to Miss Lois Lillick for qualitative and quantitative analysis of the phytoplankton. Other fractions of the water samples were bottled for determination of salinity, and of phosphate and nitrate content. The authors are indebted to Mr. Alfred Woodcock, Mr. Bostwick Ketchum, and Mr. Homer Smith, for these chemical analyses.

For the work at Station 3 from October, 1935 to September, 1936, the zoöplankton was taken in "oblique" hauls in which the net was lowered to 30 meters (just over the bottom), towed for 1 minute, then raised 1 meter and towed for 1 minute, then raised another meter and so on, until the surface was reached. A double-action hand plankton pump was added to our equipment in order that the eggs, naupliï, and younger copepodid stages of the copepods, which were too small to be retained by the scrim net, might be caught whenever spawning took place. The free end of the hose was attached to the cable about 2 meters above the weight and then lowered to the bottom. The outflow from the pump was piped directly to a 1″ Hersey Water Meter and then discharged into the top of a No. 20 silk phytoplankton net hung vertically in a metal barrel. Ten gallons (about 38 liters) were pumped at each meter from the bottom to the surface.

At 30, 15, and 0 meters bottles were filled from the hose for the chemical analyses and for quantitative phytoplankton counts by Miss Lillick. At 20, 10, and 0 meters the pump was stopped and the contents of the silk net drawn off and bottled separately, thus dividing the catch into three parts representing three strata in depth. On each occasion temperatures were taken and light penetration was measured as is described elsewhere (Clarke, 1938). When time

permitted, a second oblique zoöplankton haul and pumping operation were carried out to compare with the first. The net hauls and the pump catches were analyzed by suitable dilution and subsampling (Clarke, 1933). Our thanks are due Mr. David Bonnet for assistance in this task and in the preparation of the tables and diagrams.

General Features of the Zoöplankton

The number of species of zoöplankton represented in our catches by one or more specimens is enormous (*cf.* Fish, 1925)—too numerous for detailed analysis in an investigation devoted primarily to the

Table I

Total number of Calanus finmarchicus in hundreds calculated on a basis of a 30-minute haul.

Date 1935	Station 1	Station 2	Station 3	Station 4	Station 5
June 24	6	106	90	—	—
July 1	12	65	142	5	37
5	8	90	109	—	0
8	x	91	—	x	15
11	x	108	216	0	4
15	11	152	123	—	—
17	14	74	124	x	x
22	6	20	209	—	x
25	14	288	147	x	x
29	9	529	267	5	3
Aug. 1	84	385	232	3	0
5	5	52	171	6	6
9	11	25	72	—	—
12	3	92	94	x	x
19	8	137	50	x	0
26	0	28	113	0	0
Average	13.6	140.1	143.9	3.1	7.2

— indicates station omitted.
x indicates less than 100.

study of the production of a few important forms. Even a cursory glance at the material, however, reveals that a considerable difference exists in the plankton at the stations near shore and at those farther out. At Stations 1, 4, and 5 large numbers of larval forms of both bottom-living and pelagic species were encountered mixed in with a varying number of mature individuals of truly planktonic types. The composition of this inshore population changed almost daily in contrast to the more gradual fluctuations which took place offshore.

At Stations 2 and 3 fewer types were found in the plankton and,

although quantities of immature specimens appeared at certain seasons, these belonged mainly to the same species as the adult individuals. The difference in the population offshore is well illustrated in Table I, in which the total numbers of *Calanus finmarchicus* at each station are set forth. *Calanus* was consistently scarce at Stations 1, 4, and 5 and, although the average number at Station 3 was only slightly greater than that at Station 2, a population of at least moderate dimensions was always to be found at the offshore station.

TABLE II

Total zoöplankton taken in scrim net at Station 3, 1935–36. Thirty-minute oblique hauls with 75 cm net. Approximate volumes after settling one month.

Date	Haul No.	Vol. cc.	Av.	Date	Haul No.	Vol. cc.	Av.
Sept. 3	181	<10		May 25	204	240	
	182	<10	<10		205	160	200
Oct. 1	185	105		June 11	206	270	
	186	70	88		207	215	246
Nov 2 (Sta. 2)	187	215		June 25	208	105	
	188	160	188		209	140	123
Dec. 1	189	140	140	July 10	210	70	
Dec. 29	191	105 S			211	90	80
	192	160 S	134	July 22	212	45	
Jan. 27	193	105 S			213	40	43
	194	185 S	145	July 30	214	240	240
Feb. 23	195	700 S	700	Aug. 6	215	210	210
Mar. 27	197	10	10	Aug. 14	216	70	
Apr. 13	198	40			217	70	70
	199	65	53	Aug. 20	218	210	
Apr. 25	200	115			219	160	185
	201	115	115	Aug. 31	221	70	
May 11	202	65			222	40	55
	203	90	78	Sept. 13	223	<10	
					224	10	10

Note: Hauls mostly copepods except those marked "S" which were mostly sagittæ. The net used catches effectively animals as large as, or larger than, copepodid Stage IV of *Calanus finmarchicus*. Many smaller forms are not retained by the net.

The seasonal variation in the total population may be traced from the approximate volumes of the zoöplankton taken at Station 3 throughout the year (Table II). Plankton was scarce during September and October, but increased in November and December. The mid-winter hauls were characterized by large quantities of sagittæ. After this plankton again became scarce, but during the spring months volumes increased reaching a maximum early in June

The plankton remained abundant through the summer with few exceptions until in September a sudden reduction was encountered.

At all the stations Crustacea—usually copepods—formed the bulk of the catch with the exception of a few occasions on which large numbers of medusæ or, at the offshore stations, of sagittæ were encountered. The most common copepods, in addition to *Calanus finmarchicus*, which will be treated in a separate section, were the following (*cf.* also Fish, 1925):

Centropages typicus	July to Dec.	Numerous in August
Centropages hamatus	May to July, and in Sept. and December	Numerous in June and July
Pseudocalanus minutus	Jan. to Oct.	Numerous Mar. to Aug.
Paracalanus parvus	July to Sept. and in Feb.	Numerous in Aug.
Acartia tonsa	April to Dec.	
Oithona similis	April to Sept. and probably throughout the year	
Labidocera æstiva	Oct. to Dec.	

Many of these copepods appear to breed in this region because immature specimens of most of the list were found on one occasion or another. The copepodid stages of *Centropages* (species not determined) were particularly numerous during the summer months, and the locality is a veritable nursery for *Pseudocalanus* as judged by the large numbers of eggs, nauplii, and copepodites taken in the pump catches, particularly in the spring.

Sagitta elegans was represented by at least a few specimens in almost every haul at Station 3 and the large catches of sagittæ reported on several occasions consisted entirely of this species. *Sagitta enflata* occurred in small numbers in October and November, 1935, and in August, 1936. *Sagitta serratodentata* was taken from September to December, 1935, and in August, 1936, and was abundant on only one occasion. It was noticed that the size of *Sagitta elegans* varied greatly from month to month and that the smallest individuals appeared in January, May, July, and September. Since these months agree almost exactly with the four periods of the year during which Russell (1932–33) believes the main breeding of *Sagitta elegans* to take place at Plymouth, we may conclude that the seasonal production of this species is approximately the same on both sides of the Atlantic.

The Production of Calanus finmarchicus

Comparison of Net and Pump Catches

The numerical analysis of our zoöplankton hauls was limited almost entirely to *Calanus finmarchicus*. The numbers of this species taken in the scrim net ran into the tens of thousands. If we assume 100 per cent

straining efficiency, the scrim net filtered 564,000 liters of water during the standard 30-minute haul. In the standard pump operation, on the other hand, the total volume of water delivered to the phytoplankton net in pumping from 30 meters to the surface amounted to 1134 liters. One copepod in the pump catch would theoretically correspond to about 500 copepods in the net haul provided that the two methods are equally effective in catching all stages of *Calanus*. This was not expected to be the case, but since a few of the older copepodites and adults were usually to be found in the pump catches, these older stages were counted as well as the younger stages in order that the sampling efficiency of the pump and the net might be compared.

The total number of each stage of *Calanus* in the pump catches throughout the year is shown by the solid areas in Fig. 4. All the nauplius stages have been lumped together because the duration of each of these stages is short compared to the intervals between our observations. Very few of the earlier nauplius stages were taken. Certain eggs, which may have been those of *Calanus*, were found, but the number of these was so trifling as not to be worth plotting. The scarcity of eggs and early naupliï suggests that the actual spawning ground may be farther offshore than our station.

The numbers of *Calanus* taken in the net hauls is indicated by the superimposed single line (Fig. 4), but using a different scale. The magnitude of the scale which has been used for the net hauls was determined by finding the ratio between the two sets of data for copepodid Stage IV. This stage was chosen for the comparison of the numbers taken with the pump and with the net because it seems to have been caught effectively by both methods. Since the number of *Calanus* in this stage was high both on July 30 and August 6, the average number taken by the net on these two days was divided by the average number taken with the pump. The resulting quotient was very nearly 100. Accordingly all the totals for the net hauls were divided by 100 before plotting. The scrim zoöplankton net should theoretically remove animals from 500 times as much water as the pump. Since the volume of water passing through the pump was accurately measured, we reach the conclusion that the straining efficiency of the scrim net is 20 per cent.

The two curves for Stage IV which have been brought together at one point in the manner described are seen to correspond very roughly for the rest of the year. For copepodid Stage V and for the adults, however, the curves for the pump catches consistently lie below those for the net hauls. This indicates that the latter method is relatively more effective than the former for these larger individuals either

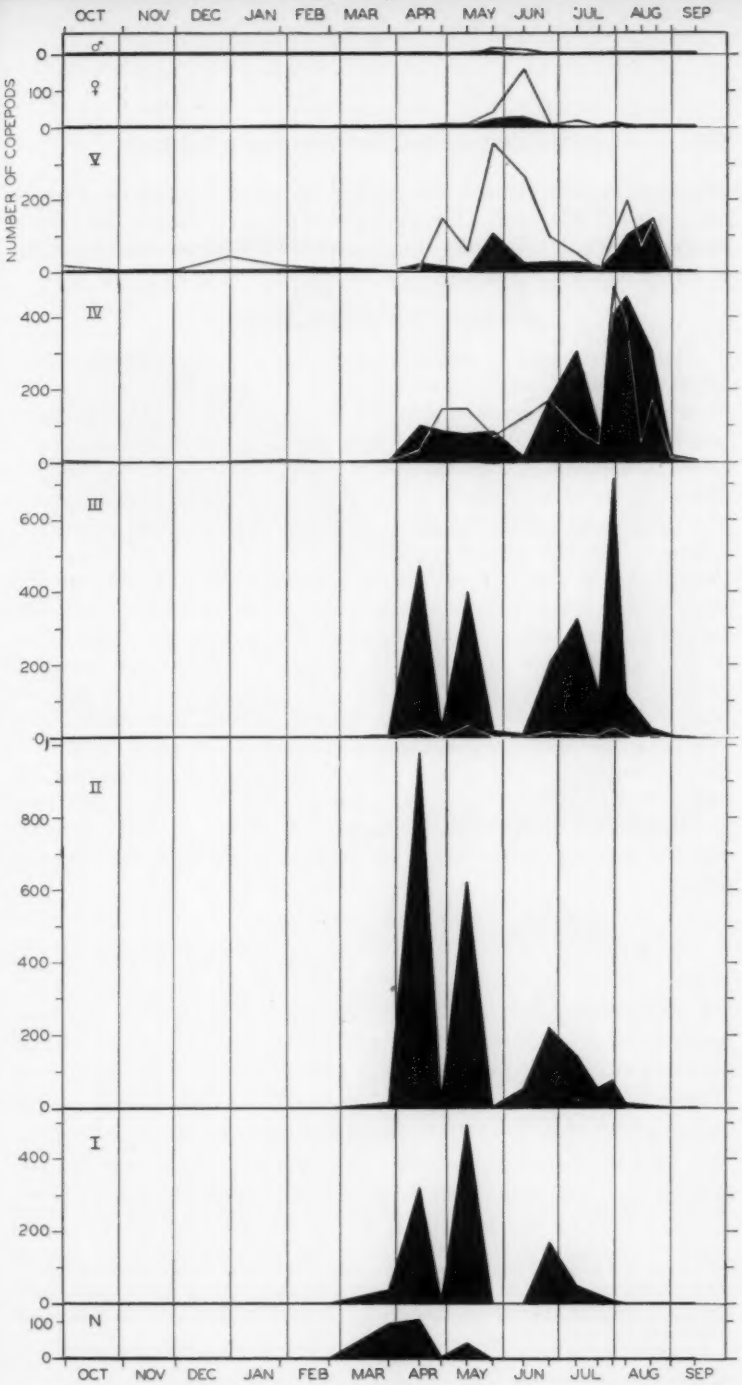

FIG. 4. The solid black area indicates the total numbers of *Calanus* nauplii (N), copepodid stages I–V, and adult ♀ and ♂ taken with the pump per standard haul (see text). The superimposed line shows the numbers of *Calanus* taken with the scrim net per standard haul, but for these the scale must be multiplied by 100.

because fewer slip through the mesh of the net or because more escape the mouth of the hose. The opposite situation is seen in the case of copepodid Stage III in which it appears that a disproportionate number of this group fails to be caught by the net.

Seasonal Changes in Abundance

Nauplii occurred in significant numbers on only three occasions, namely March 27, April 13, and May 11. Copepodid Stages I and II have peaks of abundance on April 13, May 11, and June 25. Stage III was numerous also on April 13 and May 11 and a third period of abundance extended from the end of June to the first part of August. The numbers of individuals in Stage IV were moderate in April and May, increased rapidly in June, and reached a maximum early in August. The net caught representatives of Stage V in every haul throughout the year, but with the exception of December 29, numbers were extremely low from September until the end of April. This stage was particularly abundant at the end of May and again in August, but never equalled the maximum of Stage IV. Adult specimens of *Calanus* were most numerous in May and June, but at all times were relatively scarce, particularly the males. Taking the species as a whole, we may conclude that the population is of very small dimensions during the autumn and winter. By the first of April reproduction has begun and from then on through the summer *Calanus* is abundant. At some time after the end of August the species suddenly becomes depleted and the population is not restored again until the following spring.

Succession of Generations

To determine the number of generations[3] which succeed one another during the year recourse is best made to the percentage distribution of the various age stages within each of the hauls from season to season because this procedure removes the confusing effect of fluctuations in the size of the total catch and allows the progression from stage to stage to stand out in relief. The percentage diagram for the pump catches (Fig. 5) shows that on the first of October the total catch

[3] The term "generation" is used here instead of "brood," which has been employed by others in this connection, because the former word expresses correctly the relationship of the two groups of copepods which appeared during the course of the year. The term "brood" should be limited to its strict sense in order to avoid confusion. Nicholls (1933) regards it possible for each female copepod to produce more than one brood of ova. Accordingly there may be circumstances in which the young of the second generation exist contemporaneously with a late second brood of the first generation. The necessity for using these terms with their exact meanings thus seems obvious.

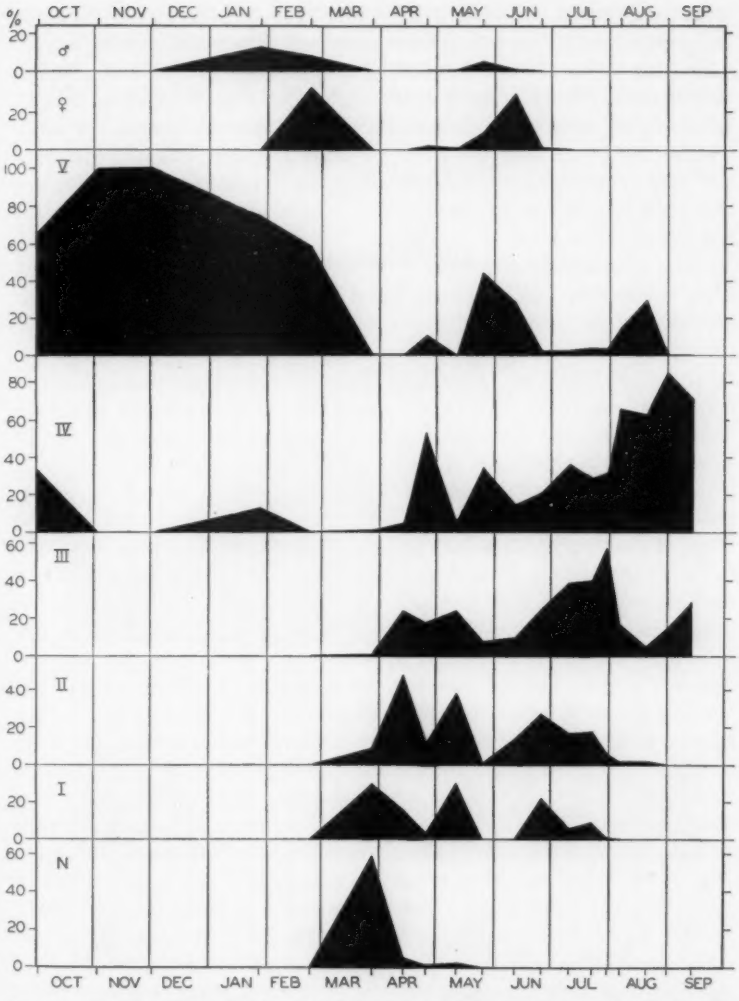

FIG. 5. Percentage distribution of *Calanus* nauplii, copepodid stages I–V and adult ♀ and ♂ taken with the pump at Station 3 during 1935–6.

consisted of 66 per cent Stage V and 34 per cent Stage IV. During November and December practically 100 per cent of the species occurred as Stage V. But in January some of these individuals matured into adult males and in February an even larger proportion of

females appeared. A month later the males and females had largely disappeared and Stage V was reduced to 1 per cent, while nauplii comprised 60 per cent of the catch and copepodid Stage I amounted to 30 per cent. During April other copepodid stages appeared in succession, but these gave rise to only a very small percentage of adult females and practically no males. In May a second series of peaks is to be found progressing up through the copepodid stages and culminating in a peak for the adult males on May 25 and a prominent peak for the females on June 11.

The relationship between these two series of peaks in April and May is obscure. Although we know that in other areas two or more generations may follow one another in rapid succession during the spring and summer, the time interval between our two series appears to be too short for them to represent successive generations. In laboratory experiments Nicholls (1933) found that 27 days was the minimum time possible from the shedding of the ova to the appearance of the adults at the temperature at which he worked (apparently 11–14° C.). The observations of Ruud (1929), Lebour (1916) and Fish (1936) indicate that a longer time than this is required for the entire development of *Calanus*. Marshall, Nicholls, and Orr (1934) state that "the eggs appear after two to four weeks, spawning may last for several weeks, and the adults then die out." Taking fourteen days as the minimum time for the maturation of the eggs and 27 days as the minimum for development, it appears that 41 days is the shortest interval which can exist between the spawning of one generation and the spawning of the next—or between the two generations at any corresponding stage of development. At certain stages in our April and May series the peaks are less than 30 days apart and the temperature of the water at the time was only about 6–8° C. It seems very doubtful, therefore, that the copepodites appearing in May were produced by the individuals which were found as copepodites in April. It is possible that the May group is a late second brood from the same parents as the April group. Adult specimens of *Calanus* were present in the water throughout April although their numbers were low. Another possible explanation of the situation is that at the end of April an unusually extensive movement of water along the coast swept away the population which had reached Stage IV on April 25 and brought in an entirely distinct population of copepods whose development was two or three weeks behind that of the first group. The reduction in the abundance of the first group when it reaches Stage V and its practical disappearance thereafter support this idea (*cf.* Fig. 4).

Another group of Stage I copepodites appeared late in June and

continued into July, but in lesser numbers. In the succeeding weeks the older copepodid stages became numerous one after another until by August 20 a prominent peak had been built up in Stage V. This summer generation was undoubtedly produced by the individuals which we observed as mature adults in May and June, although the

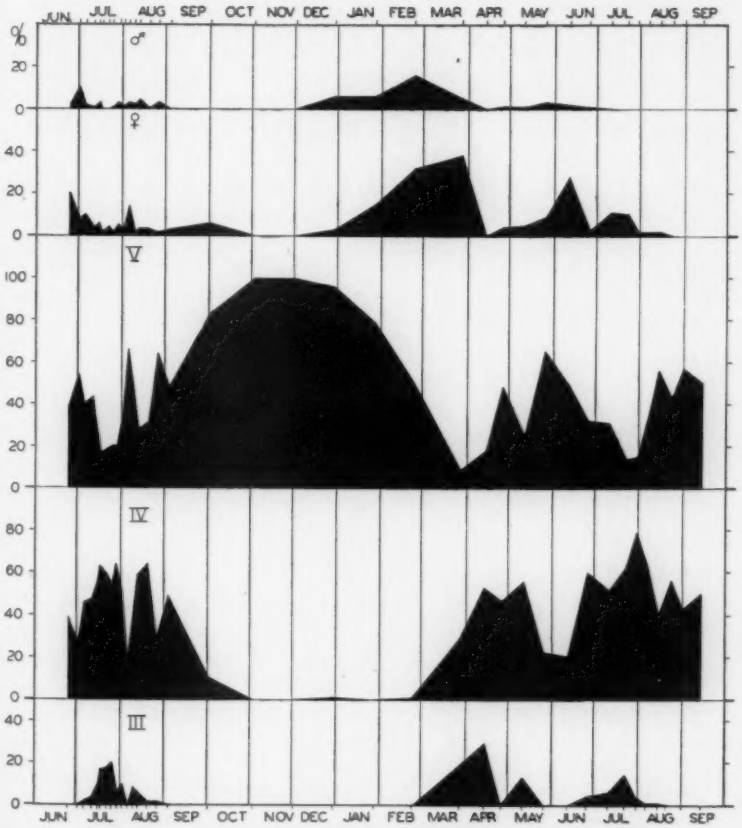

FIG. 6. Percentage distribution of *Calanus* copepodid stages III, IV, and V and adult ♀ and ♂ taken with the scim net at Station 3 during 1935–6.

eggs and nauplii which should have appeared in June were not found. Evidently the spawning took place during the two-week's interval between our visits to the station in June, or else at a point farther offshore. There is some indication in the diagram that this summer

generation consisted of more than one brood, but the irregularities were so great that no definite conclusion can be reached. It seems clear, however, that Stage IV moulted only slowly into Stage V during August and that these individuals did not mature into adults immediately, but formed the population destined to carry over the autumn and winter as Stage V.

The catches with the scrim zoöplankton net have similarly been calculated on a percentage basis to serve as a check on the conclusions reached with the pump catches (Fig. 6). The numbers of the older stages of *Calanus* taken with the pump were low and the possibility existed that the larger individuals escaped the 1-inch opening of the hose, whereas loss from this cause would be much less in the case of the scrim net. Since the net hauls were begun four months earlier than the work with the pump, we have a record for two summers and the intervening winter. At the end of June, 1935, relatively large numbers of adult males and females were found. The spawning of these produced the summer generation which appeared as Stage III copepodites in July. This generation formed the winter stock which did not mature until January, February, or March. Individuals in Stage III appearing in the net hauls in April (with a subsidiary group in May) constituted the spring generation of 1936. This generation matured and spawned in June and we find the summer generation as Stage III in July, thus nicely confirming the net haul results of the previous year.[4] As before, these individuals grew slowly during the summer, and when work was discontinued in September, the population was about equally divided between Stage IV and Stage V.

From both the pump catches [5] and the net hauls we conclude that at Station 3 a short-lived generation of *Calanus* occurs during the spring and is followed by a long-lived generation which has its origin in the early summer and lasts through the following winter. One or more subsidiary groups were found during the spring and summer which may represent second broods from the same adults, or immigrants in a different stage of development, or possibly additional generations of very short duration.

These two main breeding periods correspond closely with those observed by Fish (1936) in the Gulf of Maine for the "western stock"

[4] During the summer of 1937, however, incidental observations showed that the *Calanus* population was extremely small in this region and consisted mostly of Stage V. Evidently annual variations may be considerable.

[5] The fact that the pump catches obtained through a 1-inch hose gave the same general picture of the production of *Calanus* as the net hauls suggests that a device with a relatively small opening is adequate for plankton studies and could be more widely used (*cf.* Hardy, 1936). Such a device would have the important advantage that the volume of water strained could be accurately measured.

of *Calanus*. Fish believes that this stock forms the principal source of supply for the Gulf and he reports that these animals breed chiefly in March–April and in June–July, but possibly also in September. The developmental period of two and one-half months which Fish calcu-

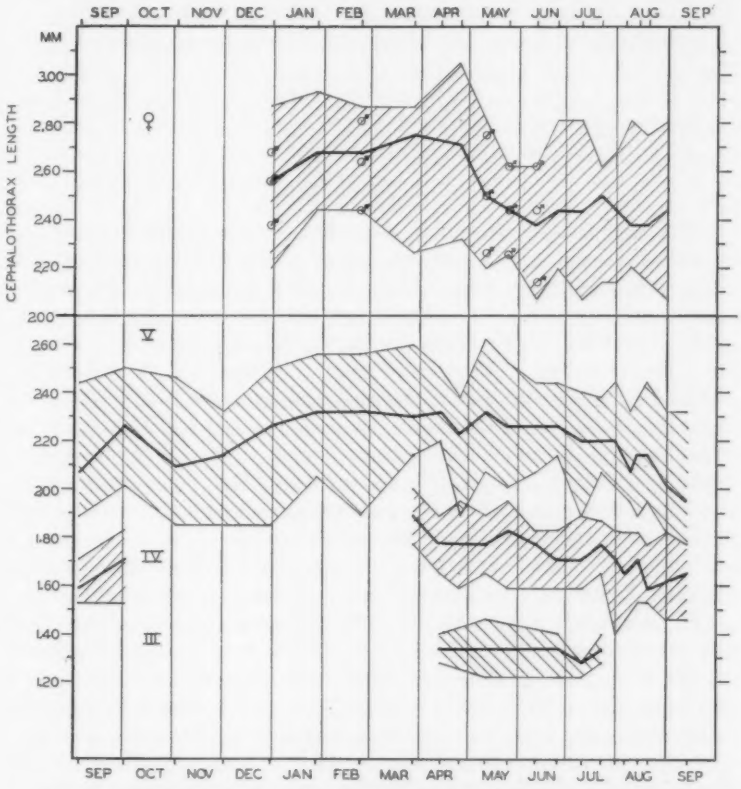

FIG. 7. Cephalothorax length of *Calanus* copepodid stages III, IV, and V and adult ♀ at Station 3 during 1935–6. The shaded area indicates the range and the heavy line the mean of 25 measurements. Since males were sufficiently numerous to measure on only five occasions, the limits of the range and the mean are indicated by the ♂ symbols placed on the upper diagram. It will be noted that the scales of the upper and lower diagrams overlap to a certain extent.

lated for his *Calanus* agrees well with the approximate interval between our spring and summer generations. Marshall, Nicholls, and Orr (1934) found that in the Clyde Sea three generations of *Calanus* occurred during the course of a year. The spawning periods for these

generations took place in March, May, and June. Our observations differ only in that the batches of nauplii appearing off Woods Hole from March to May seemed to belong to the same generation. Bogorov (1934) reports that three generations occur in the Plymouth area. He believes spawning to take place in February–March, May, and intermittently from June to August. Other investigators (Paulsen, 1906; Bigelow, 1926; Farran, 1927; Russell, 1928; Ruud, 1929; and Sømme, 1934) have found a similar succession of two or more generations in their respective areas, but since thorough reviews have been made by Fish (1936) and by Nicholls (1933), their results will not be discussed here.[6]

Variation in Size

The cephalothorax length of 25 specimens of copepodid Stage V was measured for each haul throughout the year and for Stages III and IV and for the adults on those occasions when these groups were sufficiently numerous (Fig. 7). The range of the measurements for each group in each haul was greatest for the adults and progressively less for the younger stages. The average length of Stage V did not fluctuate widely but was found to be somewhat greater from January to May than during the rest of the year. Stage IV similarly diminishes in size after the spring months have been passed. The adult males and females averaged about 2.65 mm. in length from January through April, but in May a sudden drop occurred to about 2.45 mm. and this smaller size persisted through the summer.

The abrupt reduction in the size of the adults in May coincides with the appearance in the hauls of the mature males and females of the new spring generation (Figs. 4 and 5). These individuals were spawned at the end of April or early in May when the temperature ranged between 5° and 9° C. We have already seen that the generation which was produced in June at temperatures of 11° to 15° C. apparently did not reach maturity until the following January or February. Consequently, the summer generation resulted in larger adults than the spring generation although the latter group was spawned at a temperature about 6° C. lower. This relationship with temperature is just the reverse [7] of that reported by Marshall, Nicholls, and Orr (1934) and by Bogorov (1934). These investigators found that the individuals of the spring generation were the largest of the year and Bogorov reported

[6] Results of a similar nature have been reported by Wimpenny (1936) in a publication which appeared since this paper went to press.

[7] The adult females which were taken in April and which were of large size may possibly have been spawned in March and, if so, present a case of correlation of large size and low temperature during the spawning period.

that the females of this generation were 1.2 times larger than those of the autumn-winter generation.

Comparison with Phytoplankton and Nannoplankton

The phytoplankton obtained in the water samples taken at 2, 15, and 30 meters on each occasion has been analyzed by Lillick (1937). She reports that during the summer of 1935 the phytoplankton was dominated first by *Chætoceros* and associated forms and later by *Rhizosolenia*. On October 1 the diatoms reached a maximum.[8] During the rest of the autumn and early winter the phytoplankton was extremely poor. Late in January numbers increased somewhat but by the end of February the phytoplankton was scarce again. On no occasion was a typical spring diatom maximum encountered but Miss Lillick cites evidence for believing that a great flowering of diatoms took place early in February at a time between two visits to the station. During the late spring and summer of 1936 the phytoplankton remained at a low ebb, with *Guinardia* appearing as the dominant form, until August 9 when for a period of four days a flowering of *Rhizosolenia* and *Guinardia* occurred.

Diatoms were the most prominent element of the flora at all times in this region, but dinoflagellates appeared in smaller numbers throughout the year especially in summer and fall. In addition Miss Lillick lays stress upon a group of flagellated and pigmented forms which appeared in all the samples in very large numbers. Although this group was made up entirely of exceedingly small forms, she states that their frequency was so great as to make them significant in the phytoplankton population and she believes that "they must doubtless play an important part in the general food cycle of the region."

Special water samples for the study of the bacterial population were taken by Dr. C. E. Renn at 5-meter intervals from the surface to the bottom on each trip to Station 3 from October 1, 1935 to May 26, 1936. The numbers of cells per cc. as determined by plate counts ranged from 30 to 350 during the first three months but from February to May no counts greater than 33 were obtained (Renn, 1937). Although the actual numbers of bacteria in the sea may have been a thousand times greater than this, we have shown elsewhere that populations of these dimensions cannot be a significant source of food for *Calanus* (Fuller and Clarke, 1936). Besides the bacteria, and the pigmented forms

[8] Our observations on the zoöplankton were not made at sufficiently short intervals to determine conclusively whether or not this flowering coincided exactly with a marked reduction in the number of grazing animals (*cf.* Harvey, Cooper, Lebour, and Russell, 1935). In 1935 a sharp drop in the number of *Calanus* occurred on Nov. 2; in 1936 a similar diminution of numbers took place on August 31.

mentioned above, to which Miss Lillick confined her attention, other nannoplankton types undoubtedly existed—possibly in significant quantities. The presence of great numbers of these, during the summer months at least, has been demonstrated by Lackey (1936). In most routine investigations many of the smaller and more delicate forms are probably overlooked because the usual preservatives destroy them or render them unrecognizable.

Since Marshall, Nicholls, and Orr (1934) found that in Loch Striven the periods of diatom increases coincided with the three main spawning periods of *Calanus*, it is interesting to examine our data for similar correlations. For this purpose observations should have been taken at much shorter intervals than was possible in the present

TABLE III

Phytoplankton at Station 3, March to June, 1936. The number of cells per liter at 2, 15, and 30 meters have been averaged.

	Mar. 27	Apr. 13*	Apr. 25	May 11*	May 25	June 11	June 25
Diatoms	33	9,450	600	4,700	1,467	2,300	700
Dinoflagellates	67	100	1,300	0	67	0	3,767
"Flagellates"	300	5,750	1,433	950	2,633	7,533	6,767
Other forms	0	350	0	0	67	100	167
Total	400	15,650	3,333	5,650	4,234	9,933	11,401
Calanus							
Nauplii	Abundant	Abundant		Abundant			
Copepodid I–III		Abundant		Abundant			Abundant

* Average of two depths only.

investigation. Conclusions are also made difficult in our case because of our lack of information in regard to the state of the fauna and flora in contiguous areas and the effect which horizontal movements of water masses would produce. It is clear, however, that whatever diatom flowering may have occurred in the early spring months between two visits to the station, this could not have served as a food supply for our *Calanus* since nauplii did not appear until March 27. On this date and throughout the ensuing four months diatoms were not abundant. Similarly no production of *Calanus* was observed at the times of the secondary diatom maximum in August or October. The phytoplankton which did exist during the breeding periods of *Calanus* may be studied from Table III. Diatoms were relatively numerous on April 13 and May 11, occasions when nauplii and early copepodites were abundant,

but they were scarce on March 27 when the first nauplii of the season appeared and on June 25 when the early copepodites of the second generation came into prominence. On this latter date dinoflagellates were especially numerous. The forms designated as "flagellates" were abundant throughout this period.

Although our data are not sufficient to allow us to conclude which are the essential food organisms for *Calanus* nor in what quantity they are required, nevertheless we have obtained certain facts which limit the possibilities. We have seen that the spawning periods of *Calanus* occurred at times which did not coincide with diatom maxima. We know from Miss Lillick's analysis that throughout the year the diatoms and dinoflagellates appeared suddenly at intervals of a week or two, flourished for a few days only, and then disappeared again. The "flagellates," on the other hand, although small in size, were found in relatively large numbers on all occasions. If *Calanus* feeds chiefly on diatoms, we must conclude either that the small number of cells always present as a minimum is sufficient for their nutrition, or that the food obtained by the copepods at times of local flowerings can be converted into reserve tissue (e.g. oil) which will tide them over until the next period of diatom abundance. The number of diatoms present between flowerings seems too low to fulfill the nutritive requirements of *Calanus* (Fuller and Clarke, 1936; Fuller, 1937) but further investigations may show this not to be the case. As regards the second possibility, we know that *Calanus*, in the later copepodid and adult stages at least, can live for a week or two without food, but is unable to moult successfully under these conditions (*loc. cit.*). The copepods might thus be able to survive without moulting from flowering to flowering—and this may be the actual situation during the autumn and winter—but rapid growth such as took place from April to July would probably be impossible unless adequate nutriment was continuously available. A third possibility which our present observations suggest is that the nannoplankton is important as food for copepods. The "flagellates" which were found at Station 3 at all times in large numbers would represent only a small amount of substance because of their minute size, but if, as seems likely, these pigmented forms are only a small fraction of the total nannoplankton, the latter may turn out to be significant as a food source.

Summary

1. The seasonal production of zoöplankton, particularly of *Calanus finmarchicus*, was investigated by means of scrim nets and a plankton pump at five stations in the vicinity of Woods Hole during the summer

of 1935 and at one offshore station throughout the ensuing year. Collections of phytoplankton and of nannoplankton and measurements of temperature, salinity, phosphates, nitrates, and illumination were carried out at the same time.

2. The zoöplankton consisted largely of copepods, but medusæ, sagittæ, and, at the stations near shore, larvæ of both benthonic and planktonic forms occurred irregularly. *Sagitta elegans* exhibited four main breeding periods during the year.

3. The numerical analysis of the *Calanus* population revealed the presence of nauplii in significant numbers in March, April, and May, and of early copepodites in April–May and June–July. Stage V was found at all seasons. Adult specimens were relatively scarce at all times, but most abundant in May and June. The species as a whole was reduced to small numbers during the autumn and winter.

4. The pump catches and the net hauls agree in indicating that a short-lived generation of *Calanus* occurs during the spring and that this is followed by a long-lived generation. The latter has its origin in the early summer, passes through the autumn and winter as Stage V, and matures early the following spring to give rise to the next short-lived generation.

5. The measurement of the cephalothorax length of 25 specimens in each stage older than copepodid Stage II showed that the average lengths of the various groups did not fluctuate widely during the year except in the case of the adults which exhibited a sudden drop in size in May.

6. The spawning periods of *Calanus* did not occur at times of diatom maxima, and therefore the two phenomena are not directly related in the present case. In regard to the nourishment of this copepod we must conclude (*a*) that the small number of diatoms always present as a minimum is sufficient, or (*b*) that the animals build up sufficient reserve on occasions of small local flowerings of diatoms, which occur at intervals of a week or two, to tide them over the intervening periods, or (*c*) that "flagellates," which were found continually in large numbers, and other types of nannoplankton are important as a food source.

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SEASONAL STUDIES OF THE PHYTOPLANKTON OFF WOODS HOLE, MASSACHUSETTS[1]

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During the summer of 1935 certain investigations of the plankton in the waters near Woods Hole, Massachusetts, were undertaken by workers at the Oceanographic Institution. The zoöplankton studies have been described in the preceding paper by Clarke and Zinn (1937); the observations on phytoplankton form the subject of the present paper. At the outset five stations were established for regular observations, four in Vineyard Sound, and one in Buzzard's Bay. For the phytoplankton work water-bottle samples were taken at each station twice weekly, and at three depths, surface, mid-depth, and bottom. Along with these, determinations of the temperature, salinity, nitrate and phosphate content of the water were made.

During the first two months the water at all five stations was found to be so homogeneous in nature with regard to the phytoplankton, that it seemed unprofitable to continue observations at all stations. Therefore all subsequent collections were made at Station 3 alone, which is located at the Whistle Buoy near the western entrance to Vineyard Sound, Latitude 41° 17′ 35″, Longitude 71° 0′ 0″ (see Clarke and Zinn, 1937, for map). This station was chosen because conditions there, more nearly than at any of the others, simulate those of open waters, as will be seen later. From September, 1935, through the winter months samples were taken once a month; from April through July, fortnightly; during August, weekly. The final phytoplankton data were collected on August 20, 1936, giving a range of fourteen months for the entire survey. Samples were taken at the 2-meter, 15-meter, and 30-meter levels, the bottom being at 32 meters.

In this paper only such forms have been included as fall naturally within the plant classes, the chlorophyll-bearing or food-producing groups, which include the *Cyanophyceæ*, *Diatomaceæ*, *Dinoflagellatæ*, *Silicoflagellatæ*, *Coccolithineæ*, and certain of the pigmented flagellated groups. All of the true protozoan classes have been omitted.

In making the quantitative determinations of the phytoplankton

[1] Papers from the Department of Botany of the University of Michigan, No. 629; Contribution No. 156 of the Woods Hole Oceanographic Institution.

for the majority of the samples Gran's centrifuging method was used, modified somewhat by having the organisms concentrated over a membrane filter, and then centrifuged. Since by this method others have found that there may be a 10 per cent loss of organisms, an original sample of 1,100 cc. was used instead of 1,000 cc. to allow for this error. For the winter and spring samples the precipitation method of Nielsen and von Brand (1934) was used chiefly because the amount of sample available was not sufficient to permit the use of the other method.

Most of the collections were made by Mr. Donald Zinn and the crew of the Oceanographic Institution boat *Asterias*. Chemical determinations were made by Dr. Homer P. Smith and Mr. Bostwick Ketchum. The writer is indebted to Dr. H. B. Bigelow and Dr. F. K. Sparrow, Jr., for certain guidance at the Woods Hole Oceanographic Institution, and to Dr. William R. Taylor and Professor H. H. Bartlett under whose direction the work was completed at the University of Michigan.

Hydrographic Observations

Certain physical features of the water, currents, tides, temperature, and salinity, play an undoubtedly important part in the distribution of plankton. The observations made on these factors during the present investigation have been presented by Dr. Clarke (Clarke, 1937; Clarke and Zinn, 1937). Although the control of phytoplankton species distribution, at least in part, must be attributed to the influence of temperature and salinity, no obvious correlations could be made between the variations in these factors and in the seasonal distribution of phytoplankton.

Nitrate determinations ranged from 4 milligrams of N. as NO_3 per cubic meter of sea water at 15 meters on October 1, to 62 milligrams at 2 meters on December 29 (see Figs. 1, 2, and 3). Through the summer months of the first year, the nitrate content of the upper strata of water was low, exhibiting slight fluctuations. Somewhat higher values were obtained in the lower strata. During the winter nitrates were gradually built up throughout the entire water mass to their highest concentration in early January. It is noteworthy that there is a decided lag in this accumulation of nitrates behind that of phosphates, a feature which is consistent with Cooper's theory (1933) based on observations in the English Channel, that the phosphates are returned directly to the water upon the decomposition of the plankton; whereas the nitrates are regenerated only after a series of intermediate steps. Terminating the winter accumulation, there was a sudden dropping off in the amount of nitrates in late January and early February, followed by a more gradual decrease throughout March and April. The summer was marked by small amounts of nitrates with

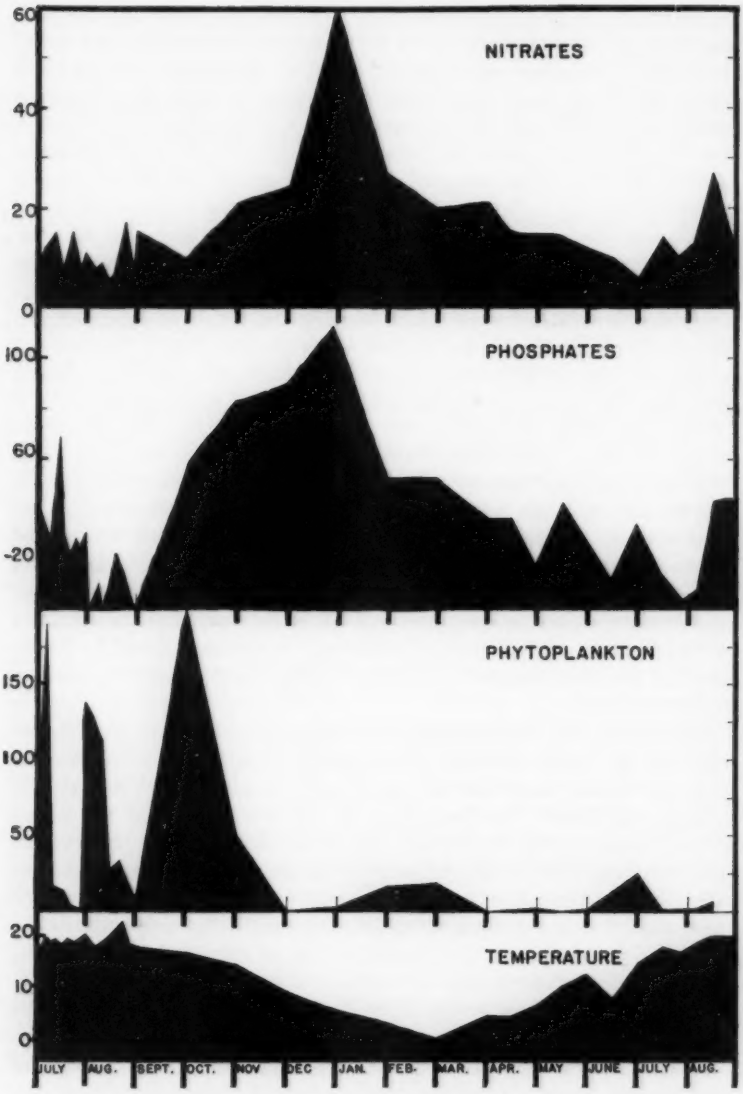

FIG. 1. Distribution of nitrates, expressed as milligrams of N as NO_3 per cubic meter of water; phosphates, as mg. of PO_4 per cubic meter; phytoplankton, in thousand cells per liter; and temperature, in degrees centigrade, at 2 meters depth, Station 3, from July, 1935, to Aug., 1936.

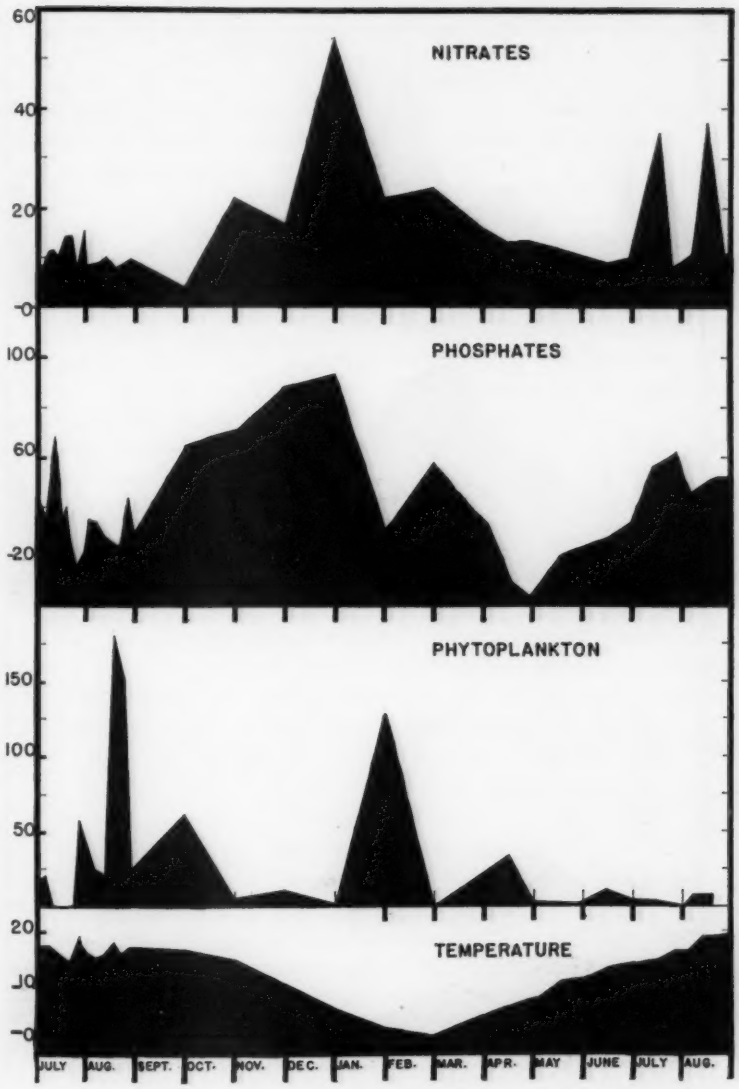

FIG. 2. Distribution of nitrates, expressed as milligrams of N as NO_3 per cubic meter of water; phosphates, as mg. of PO_4 per cubic meter; phytoplankton, in thousand cells per liter; and temperature, in degrees centigrade, at 15 meters, Station 3, from July, 1935, to Aug., 1936.

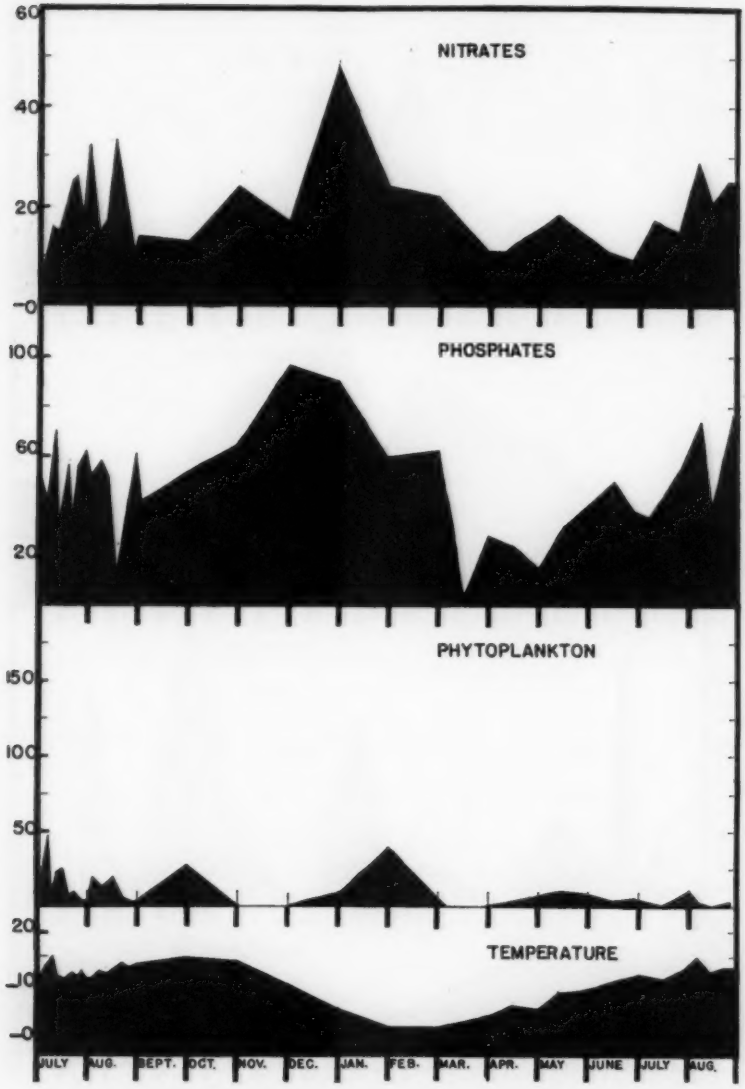

FIG. 3. Distribution of nitrates, expressed as milligrams of N as NO_3 per cubic meter of water; phosphates, as mg. of PO_4 per cubic meter; phytoplankton, in thousand cells per liter; and temperature, in degrees centigrade, at 30 meters, Station 3, from July, 1935, to Aug., 1936.

only slight fluctuations. In August there was an accumulation to form a second lesser peak, which again lagged behind the phosphates, and which was followed immediately by a decrease in concentration. Variations with depth were never extremely pronounced. However, there was usually some degree of stratification. From May through August, the nitrates tended to become concentrated at the bottom. By September they were more or less uniformly distributed, and remained so until December, when the surface waters became very rich in nitrates, the bottom waters much less so. This general relationship continued through April.

The phosphate distribution differed somewhat from that of nitrates. The total amounts found in the water were always much higher, though the total range was greater, from none in the surface waters through most of August and September, to 112 mg. PO_4 per cubic meter at the surface in December. The seasonal phosphate cycle was as follows: low through July and August of the first summer; increasing through September and October; reaching a maximum during the winter months of November, December and January; decreasing from the end of January through February and March, and dropping to the lowest point for the year in April, remaining low through May and June; in July and August gradually increasing, with another drop at the end of August. Stratification of phosphates was frequently much more pronounced than that of nitrates. From June through August the phosphates were found to be more concentrated at the bottom. In October the waters became more nearly uniform throughout, and remained so until spring, when the amounts at the surface increased over those at lower layers, this relationship remaining until June. The possible correlation of phytoplankton production with nitrates and phosphates will be discussed later.

PHYTOPLANKTON

(See Tables I and II)

The larger part of the phytoplankton in our collections was made up of diatoms, dinoflagellates, and flagellates. During certain seasons of the year members of the Silicoflagellatæ and Coccolithineæ became rather numerous, and stray members of the Cyanophyceæ appeared in the collections on occasion. More than 100 different species appeared during the year, divided among the several groups as follows:

Diatomaceæ	57
Dinoflagellatæ	31
Silicoflagellatæ	3
Coccolithineæ	2
Cyanophyceæ	2
Flagellatæ	?

TABLE I

Seasonal distribution of the more important phytoplankton species at 15 meters, Station 3, July, 1935, through August, 1936.

Species	Cells per Liter											
	July 5	July 15	July 25	Aug. 1	Aug. 12	Aug. 26	Sept. 3	Oct. 1	Nov. 2	Dec. 1	Dec. 29	Jan. 27
Diatomaceæ	....	....	....	....	....	....	....	....	....	....	....	....
Asterionella Bleakleyi	....	....	....	....	598	228	....	....	....	....	....	....
Chætoceros spp.	....	....	180	....	184	500	....	....	4,000	....	....	....
Ch. borealis	....	....	....	....	184	46	....	2,300	....	....	....	....
Ch. constrictus	....	....	1	....	138	1,496	....	500	....	....	....	....
Ch. decipiens	270	....	....	....	....	....	....	900	....	....	....	....
Ch. didymus	....	....	....	....	....	....	....	2,200	....	....	....	....
Corethron hystrix	....	....	....	138	36,708	2,048	....	....	....	....	....	....
Guinardia flaccida	....	....	....	322	1,196	....	....	100	600	200	....	....
Leptocylindrus danicus	....	....	....	....	4,874	....	....	2,300	....	700	....	....
L. minimus	....	....	....	....	42,964	....	....	300	....	....	....	....
Melosira sulcata	225	....	....	1,485	....	228	....	660	600	300	....	....
Nitzschia Closterium	90	....	46	....	3,108	228	....	....	....	....	....	....
N. seriata	135	....	....	....	14,306	368	....	17,600	3,600	5,800	....	....
Rhizosolenia alata	....	....	....	....	....	....	....	1,300	....	....	....	....
R. calcar-avis	....	....	....	138	....	....	....	....	100	....	....	....
R. fragillissima	....	....	....	....	....	....	....	800	....	100	....	....
R. hebatata semispina	270	....	46	....	....	....	....	300	200	....	....	330
Sceletonema costatum	....	....	....	506	46,782	1,035	....	30,000	....	....	....	....
Thalassionema nitzschioides	7,965	92	....	230	2,392	46	....	1,200	....	700	900	500
Thalassiosira Nordenskiöldii	225	....	....	1,196	276	405	....	800	....	300	300	3,800
Dinoflagellatæ	....	....	....	....	....	....	....	....	....	....	....	....
Gonyaulax tamarensis	90	....	....	....	322	....	1,300	....	....	....	....	....
Peridinium spp.	....	....	....	....	92	1,588	....	100	....	....	....	....
P. simplex	....	....	....	....	3,184	774	....	....	....	....	....	....
Prorocentrum minimum	6,480	92	598	3,780	2,832	2,502	....	....	....	....	....	....
P. Scutellum	....	....	....	368	966	319	....	....	....	....	....	2,160
Silicoflagellatæ	....	....	....	....	....	....	....	....	....	....	....	....
Distephanus speculum	1,755	....	....	184	....	....	....	100	....	....	....	....
Coccolithineæ	....	....	....	....	....	....	....	....	....	....	....	....
Syracosphæria	....	....	....	....	....	....	100	....	....	....	....	....
Flagellatæ	1,215	....	....	2,852	9,338	8,681	900	500	300	1,100	500	1,992
Total at 2 meters	189,000	16,000	35,000	136,000	30,000	19,400	9,300	214,000	53,000	1,000	4,900	16,000
Total at 15 meters	20,000	400	1,240	11,000	181,000	26,000	3,500	63,000	6,800	11,000	1,700	127,000
Total at 30 meters	45,000	19,000	9,800	2,900	21,000	2,070	....	28,000	500	200	7,300	39,000

TABLE I—*Continued*

Species	Cells per Liter											
	Feb. 23	Mar. 27	Apr. 13	Apr. 25	May 11	May 25	June 11	June 25	July 10	July 22	Aug. 6	Aug. 14
Diatomaceæ	....	....	....	....	....	....	....	....	....	....	....	....
Asterionella Bleakleyi	....	....	....	....	....	....	....	....	....	....	....	....
Chætoceros spp.	....	....	....	....	....	....	....	....	....	....	....	....
Ch. borealis	....	....	....	....	....	....	....	....	....	....	....	....
Ch. constrictus	....	....	....	....	....	....	....	....	....	....	....	....
Ch. decipiens	....	....	....	....	....	....	....	....	....	....	....	....
Ch. didymus	....	....	....	....	....	....	....	....	....	....	....	....
Corethron hystrix	....	....	....	....	....	....	....	....	....	....	....	500
Guinardia flaccida	....	....	18,500	....	....	....	....	....	....	300	300	1,200
Leptocylindrus danicus	....	....	....	....	....	....	....	....	....	....	....	....
L. minimus	....	....	....	....	....	....	....	....	....	....	....	....
Melosira sulcata	....	....	....	....	....	....	....	....	600	....	300	700
Nitzschia Closterium	....	....	....	....	....	....	....	....	....	....	....	....
N. seriata	....	....	....	....	....	....	....	....	....	....	....	....
Rhizosolenia alata	....	....	....	....	....	....	....	....	....	....	....	....
R. calcar-avis	....	....	....	....	....	....	....	....	....	....	700	700
R. fragillissima	....	....	....	....	....	....	....	....	....	....	....	....
R. hebatata semispina	200	....	....	....	....	....	....	....	....	....	....	....
Sceletonema costatum	....	....	....	....	....	....	....	....	....	....	....	....
Thalassionema nitzschioides	....	....	100	....	....	....	200	....	....	....	....	1,000
Thalassiosira Nordenskiöldii	....	....	....	....	....	....	....	300	500	....	....	500
Dinoflagellatæ	....	....	....	....	....	....	....	....	....	....	....	....
Gonyaulax tamarensis	....	....	....	....	....	....	....	....	....	....	....	....
Peridinium spp.	....	....	....	....	....	....	....	....	....	....	....	....
P. simplex	....	....	200	....	....	....	....	....	....	....	....	....
Prorocentrum minimum	....	....	....	....	....	....	....	200	200	....	800	100
P. Scutellum	....	....	....	....	....	....	....	....	100	....	200	200
Silicoflagellatæ	....	....	....	....	....	....	....	....	....	....	....	....
Distephanus speculum	....	....	....	....	....	....	....	....	....	....	....	....
Coccolithineæ	....	....	....	....	....	....	....	....	....	....	....	....
Syracosphæria	....	....	600	....	....	....	....	100	....	....	1,000	....
Flagellatæ	....	....	9,500	1,800	....	1,000	10,200	1,200	1,200	....	3,200	1,400
Total at 2 meters	19,000	500	2,300	2,800	200	2,500	15,600	26,000	2,500	2,600	2,800	7,150
Total at 15 meters	200	700	29,000	1,900	....	1,000	11,000	2,900	2,000	300	6,800	7,000
Total at 30 meters	....	....	....	5,700	11,000	9,200	3,300	5,900	1,300	12,000	300	800

TABLE II

Complete list of the species found at Station 3 during the survey of 1935–1936.

DIATOMACEÆ
Achnanthes tæniata
Asterionella Bleakleyi
japonica
Bacteriastrum hyalinum
Cerataulina Bergonii
Chætoceros spp.
affinis
borealis
borealis var. *concavicornis*
compressus
constrictus
curvisetus
debilis
decipiens
didymus
gracilis
laciniosus
teres
Cocconeis placentula
Corethron hystrix
Coscinodiscus centralis
excentricus
lineatus
Grammatophora marina
Guinardia flaccida
Hemiaulus Hauckii
Leptocylindrus danicus
minimus
Lichmophora abbreviata
Melosira moniliformis
sulcata
Navicula spp.
Nitzschia Closterium
longissima
seriata
Pleurosigma Normanii
Rhabdonema adriaticum
Rhizosolenia alata
calcar-avis
fragillissima
hebetata var. *semispina*
imbricata var. *Shrubsolei*
setigera
styliformis
Sceletonema costatum
Striatella unipunctata
Surirrella Gemma ?
Synedra Gallionii ?
Thalassionema nitzschioides
Thalassiosira decipiens
gravida
Nordenskiöldii
Thalassiothrix Frauenfeldii
longissima
DINOFLAGELLATÆ
Ceratium Fusus
lineatum
longipes
longipes var. *oceanicum*
macroceros
Tripos
Dinophysis acuminata
norvegica
Ovum
Exuviælla baltica
Glenodinium trochoideum
Gonyaulax tamarensis
Mesoporos asymmetricus [2]
Minusculus bipes
Noctiluca miliaris
Peridinium spp.
breve
Cerasus
conicoides
conicum
denticulatum
depressum
gracile
Granii
simplex
Phalocroma irregulare ?
Prorocentrum micans
minimum
Scutellum
SILICOFLAGELLATÆ
Dictyocha Fibula
Distephanus speculum
Ebria tripartita
COCCOLITHINEÆ
Rhabdosphæra tubulosa
Syracosphæra spp.
FLAGELLATÆ
CYANOPHYCEÆ
Glæocapsa
Oscillatoria

Both oceanic and neritic forms occurred with several important species belonging to either class. Neritic forms included both pelagic and bottom types; and occasional tychopelagic forms appeared.

The summer flora (see Table I) of 1935 was broadly typical of the area for that season. In July various species of *Chætoceros* were important. This is the normal summer flora for regions lying to the north of Woods Hole, and to be expected here. This genus was accompanied by *Corethron hystrix*, *Guinardia flaccida*, *Leptocylindrus danicus* and *Nitzschia seriata*, all temperate or boreal forms. In August these forms were replaced by species of *Rhizosolenia* (*R. calcar-avis*, *R. alata*, and *R. setigera*) and *Thalassionema nitzschioides*, a temperate flora. The summer maximum was reached during August, and consisted of *Rhizosolenia* and *Guinardia*. These are such large forms that the numbers recorded give a false impression of the actual state of the water. Plankton nets drawn through the waters during one of these maxima became clogged within a few minutes with *Rhizosolenia*. This summer maximum is very typical of the general area, and is similar to that found by Fish (1925). The entire flora

[2] Since the name *Porella* given by Schiller (1928) to a genus of the family Prorocentraceæ, Dinoflagellatæ, is identical with that of the well known liverwort genus *Porella* [Dill.] L., order Jungermanniales, and since this latter group antedates Schiller's genus by a great many years, it seems necessary to change the name of the dinoflagellate group. For this genus the name *Mesoporos* is offered here in substitution for *Porella* Schiller. Since Schiller's generic name was not established by a Latin diagnosis, one is given here for the nomenclatorial validation of the genus, in accordance with the International Rules.

Mesoporos gen. nov.

Porella Schiller non *Porella* [Dill.] L.

Cellula ovalis plusminusve lateraliter applanata. Flagella per fenestram edentatam poriferem exserta. Hemitestae poro singulo mediano intus projecto praeditae, si lateraliter viso invaginationem conicam obtusam apice perforatam formanti. Chromatiphora dua vel tria flava vel brunneo-flava ad hemitestas applanata, absque pyrenoideis. Species 5, a cl. Jos. Schiller sub nomen Porellam descriptae, Arch. f. Protistenkunde 61: 54. 1928.

Mesoporos bisimpressus (Schiller) comb. nov.

Exuviælla bisimpressa Schiller, Arch. f. Protistenk. 38: 257. 1918. *Porella bisimpressa* ibid., 61: 54. 1928.

Mesoporos asymmetricus (Schiller) comb. nov.

Porella perforata (Gran) Schiller, *Arch. f. Protistenk.*, 61: 55. 1928. *Porella asymmetrica* Schiller, Dinoflagellata, Bd. 10, 3 Abt., S. 29, in Rabenhorst's Kryptogamen-flora. 1931.

Mesoporos globulus (Schiller) comb. nov.

Porella globula Schiller, *Arch. f. Protistenk.*, 61: 56. 1928.

Mesoporos adriaticus (Schiller) comb. nov.

Porella adriatica Schiller, *Arch. f. Protistenk.*, 61: 56. 1928.

Mesoporos perforatus (Gran) comb. nov.

Exuviælla perforata Gran, 1915, Bull. plankt. for 1912. Cons. Perm. int. p. l'explor. de la Mer, Copenhagen.

for this season was much more characteristic than that found in the following summer.

The winter flora, following the fall maximum, was extremely poor (see Table I). Collections made on October 1 showed a relatively large number of diatom species, occurring in considerable abundance. These collections were very similar to Fish's (1925) typical winter flora and indicate that the season of 1935 resembled those of 1922 and 1923 at that time. In October 1935 the species which Fish had found to be dominant were again dominant, namely, *Rhizosolenia alata*, *Sceletonema costatum*, and *Leptocylindrus danicus*. Certain of the forms which he mentions as occurring in abundance were found in our collections to be important here in October and at no other time throughout the winter (*Chætoceros decipiens* and *Rhizosolenia setigera*). This similarity is surprising in the face of the total lack of similarity throughout the rest of the winter.

For the rest of the winter months our collection showed that the plankton as a whole was extremely poor. The four important species, and at times nearly the only species found, were *Thalassionema nitzschioides*, *Rhizosolenia hebatata* var. *semispina*, *Thalassiosira Nordenskiöldii*, and *Nitzschia seriata*. All are boreal forms, the first two oceanic, and the last two neritic. Nearly all of the supplementary species were likewise boreal. Along with these forms there was a relatively high percentage of flagellated forms, a group which appeared throughout the year and which will be discussed later. These results are in the main very different from those of Fish (1925), who found a definite winter "maximum" consisting of a greater variety of abundant species than the summer.

It is extremely unfortunate that the great difficulty in making these collections necessitated the spacing of the trips rather far apart. During much of the year, because of the relative stability of the flora, this should have made little difference. Nevertheless it has resulted in our failing to observe any especial spring flowering of diatoms which may have occurred and which is so characteristic of the region north of Cape Cod. It is well known from the work of Bigelow, Gran and Braarud, and others that at some time during the early spring, and for a period of short duration, there is a flowering of diatoms dominated by *Thalassiosira Nordenskiöldii* in the Gulf of Maine, Bay of Fundy, and Massachusetts Bay, which results in phytoplankton numbers far in excess of those of any other season of the year. Fish seems not to have found a similar flowering, though he mentions *T. Nordenskiöldii* as occurring throughout January, February and March. However, the conditions with which he dealt at the Bureau of Fisheries' dock

were far different from those at our more off-shore station. He does, however, record the occurrence of a winter maximum beginning in November or December and lasting into March, for Vineyard Sound and Long Island Sound. Whether or not a flowering of either type occurred at our station during the spring of 1936 must remain problematic; certainly no such conditions appeared in our collections. It is entirely possible that in the interval between any two of our spring collections such a flowering could have burst forth and disappeared again without showing up at all in our collections. The weight of evidence which we have at hand seems to indicate that if such a flowering occurred during the spring of 1936, it could not have happened in all probability later than early February. Collections taken at the end of January show that the phytoplankton had increased decidedly over the winter months, yet was not abundant enough to be considered as a "flowering." Further, there was a decided increase in the number of cells of *T. Nordenskiöldii*. Other species which began to appear or to become prominent were *Rhizosolenia fragillissima*, *Nitzschia seriata*, *Thalassiosira decipiens*, *Thalassionema nitzschioides*, and certain of the peridinians, all boreal forms of the neritic or oceanic type.

By the end of January the nitrates and phosphates in the water had decreased markedly from their winter maximum concentration in December. Similar sudden depletions of these salts have been found by others in the English Channel, Loch Striven, and elsewhere (Harvey, 1928; Atkins, 1927; Marshall and Orr, 1929) to be coincident with the outburst of spring phytoplankton. It seems logical to infer that such an explanation may account for the sudden decrease in these salts at our station. The collections of late February and the following months show a marked falling off of the phytoplankton, and of the critical species, a condition which would normally follow the spring maximum. All of these arguments combine to indicate that any spring flowering of diatoms which may have occurred at our station must have done so between the end of December and the earlier part of February.

Following February the plankton became exceedingly scarce, and entered the summer period, which for 1936 was the poorest for the entire year. A few species of *Chætoceros*, which usually make up a large portion of the summer flora in this latitude, and which were prominent the year before, appeared early in the season but died out as the year progressed. The important species throughout the season was *Guinardia flaccida*, a temperate neritic form. The flagellates also became prominent for a time in June, and then decreased. Temperate

forms became more common; whereas boreal forms tended to die out. Certain of the smaller dinoflagellates appeared during the summer, though never in great numbers. The summer maximum, which is of very short duration, did not show up in our collections. However, we do have other information which gives the time and nature of this summer flowering. Collections made in another connection at the Oceanographic Institution showed that during a period of three or four days, from August 9 to August 11 or August 12 the waters in Vineyard Sound were teeming with a diatom flora which excluded nearly everything else, coloring the water a deep olive green. An examination showed this flowering to be made up almost entirely of the two large diatom species, *Rhizosolenia calcar-avis*, and *Guinardia flaccida*, both of which were prominent in the summer maximum of the year before. This growth appeared very suddenly and disappeared just as rapidly. As can be seen from our collections of August 6 and August 14, both species were present in the waters, but in very small amounts. Table I shows the general occurrence and abundance of the more important species found throughout the fourteen months at a depth of 15 meters.

Diatoms were obviously the most important element of the flora throughout the year. Dinoflagellates appeared throughout the year, more frequently in summer and fall, but their numbers were never sufficient to outweigh the diatoms. A group of forms which were exceedingly small, flagellated and pigmented, which therefore must be considered in a treatment of phytoplankton, kept appearing in all of our collections throughout the year, and in very large numbers. The formaldehyde used to preserve the collections apparently so distorted these forms that identification was impossible. There is no question that many widely separated organisms appeared in this class, which have been lumped together indiscriminately here under the term "Flagellatæ." At times certain of the forms were obviously swarm spores of certain of the diatoms and peridinians. It is quite possible that others may have been spores of other algal groups. Some were evidently independent flagellate forms. Much difficult work will be necessary before this general group can be clearly understood. No mention of such a prominent group as we have found seems to have gotten into the literature of this general region, probably because much of the previous work has been done with a no. 20 plankton net, which would allow these small types to slip through the meshes. Poor though our general grouping of the forms may be, it seems desirable to include them in this account because they doubtless play an important part in the general food cycle of the region.

In Table II is given a complete list of the phytoplankton found to occur at Station 3, during the survey.

Broadly speaking, then, our results correspond well with those of Fish, whose work is the only one of a similar nature conducted in a nearby region. Making allowances for the great differences between the two localities, and for the limits placed upon our collections, the seasonal cycle of the two surveys corresponds remarkably well. Comparison of the species given in Table II with those listed by Fish shows that a certain number of littoral or bottom forms appeared in his collections and not in ours. A number of these species appeared in our collections made during the first summer at the more littoral stations. Other features characteristic of more offshore waters are shown by our station. Along with stratification of the water column is seen a certain degree of stratification of phytoplankton. One of the outstanding characteristics of Fish's station is the homogeneity of the water from surface to bottom. The seasonal cycle at Station 3, if we consider that we have sufficient data from which to judge, more nearly resembles that of offshore waters, with its spring and fall maxima, and summer and winter lulls, than it does the neritic waters. Conditions here must be considered to be somewhat intermediate between the other two types.

For a long time oceanographers have held to the theory that phytoplankton production is tied up with and dependent upon the amounts of nitrates and phosphates found in the water in any given latitude. In a broad sense, although this does not seem to be the final factor which controls the cycle, there must be, certainly, some close correlation between the two. Some such broad relationship can be seen in the results of our survey. An examination of Figs. 1, 2, and 3 will show that the nitrates and phosphates were relatively plentiful during July and early August of 1935, and that they dropped suddenly during August. They gradually accumulated during the fall and rose to their highest point during the winter months. During January there was a sudden drop to a summer low which continued until July, when the salts began to accumulate again to a lower peak in August. Another decrease occurred here. Considering the curves for the plankton, it can be seen that there are certain points of correlation, assuming that the spring flowering occurred, as has been indicated earlier, before the beginning of February, and the summer one in the middle of August. When the phytoplankton was low, the salts began to accumulate. As the phytoplankton began to increase, the salts dropped off suddenly, implying that they were used up in the plant cells. The dying off of the phytoplankton was again followed by a

gradual accumulation of salts. These were no doubt supplied in part by the decomposition of dead organic matter, and in part by new water entering the Sound. There are minor differences between the curves throughout, but in the major phases this general relationship holds. Nitrates seem to be somewhat more critical for phytoplankton in these particular waters than phosphates. There was always more PO_4 in the water column than NO_3, probably always enough for some plant growth. It is with the curve for NO_3 that the phytoplankton shows the greatest correlation, and it may be that only when the PO_4 curve agrees with that of NO_3 in trend that it can be considered related to the plankton production. There is a much more pronounced relationship between the phytoplankton and the salts at 2 meters and 15 meters than at the bottom, the 15-meter level showing the clearest correlation. The general ratio of nitrates to phosphates which is shown by Redfield (1934) to exist in the open oceans, and which corresponds in general to the proportions in which these occur in plankton organisms, does not hold at our station. Our data reveal that throughout most of the year the phosphates exceeded the nitrates, a condition which is contrary to that normally found.

Quantitatively, a greater number of organisms were found near the surface than at the bottom. The greatest development of phytoplankton is usually found to be in the lower strata of water, but at our particular station the amounts of detritus found in the water at all levels was so noticeably greater than that found in more open waters, that it is quite possible that throughout much of the year light is not available at the lower levels for photosynthesis. It happened very frequently that the number of cells at the bottom greatly exceeded that at 15 meters. This is probably explainable by the fact that cells sink rapidly once they become inactive, hence the active cells are those at the surface; those at the bottom have become inactive or are dead. In general the important species found at all three depths at any one time were the same. No counts obtained during the entire survey were high, the highest number recorded being 214,000 cells per liter at the surface in October, a number which is normally much exceeded during the spring maximum at other localities. Doubtless the numbers during the spring flowerings which occur in this region would exceed 200,000 many times. The lowest number recorded was 200 cells per liter at 30 meters in December, and at 15 meters in February. Collections made in March for the entire water column were lower than at any other time of year.

This survey, incomplete though it is, and lacking two important phases of the phytoplankton cycle, adds definitely to our knowledge

of the waters near Woods Hole. It is the first study of the sort in this region of a station which is far enough from land to show some of the hydrographic features of the open sea. The quantitative work gives a reasonably accurate idea of the numbers of phytoplankton cells which occur in these waters at the different seasons; and the possible relationship between the plankton and the concentration of nitrates and phosphates is indicated.

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SERUM PROTEIN MEASUREMENTS IN THE LOWER VERTEBRATES. I. THE COLLOID OSMOTIC PRESSURE, NITROGEN CONTENT, AND REFRACTIVE INDEX OF TURTLE SERUM AND BODY FLUID

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The significance of the blood serum proteins in the maintenance of the fluid balance between blood and tissues was first suggested by Starling (1), who postulated that the osmotic pressure they exert is the force which, acting in opposition to the hydrostatic pressure of the blood, prevents the loss of excessive amounts of fluid to the tissues. The numerous studies of the serum colloid osmotic pressure which have since been made in man and the lower mammals have indicated that for mammals Starling's hypothesis holds true, if not completely, at least as a major factor. A comprehensive review of the subject, with bibliography, is that of Landis (2). A recent modification of method is that of Wies and Peters (3).

The question of the applicability of this hypothesis to conditions which obtain in the lower vertebrates has been investigated very little. Data on the amphibia are given by Landis (2) and also by Drinker and Field (4). The most recent work has been that of Keys and Hill (5), who studied six species of fish. It has seemed important to us to make a series of studies of the colloid osmotic pressure of the serum in several of the lower vertebrates, correlating the findings whenever possible with other determinations on the serum proteins. In this paper we present the results of a study of a reptile, the common "slider" turtle. Determinations were made of the colloid osmotic pressure, nitrogen content, and refractive index of the serum and of the body fluid of normal animals and of those subjected to long-continued starvation at two different temperatures.

METHODS

Fourteen adult, healthy, female turtles of the species *Malacoclemmys geographica*, Les. were obtained in October at about the end of the period of summer activity and feeding. Two of these were studied at once as normal fall animals and the remainder divided into four experi-

mental groups, of which one was kept during the succeeding months under each of the following conditions: (1) room temperature, 19°–20° C., with feeding, (2) room temperature without feeding, (3) winter temperature, 7°–10° C., with feeding, (4) winter temperature without feeding. The food consisted of common garden worms *ad libitum;* all animals were kept constantly in large tanks of fresh water. During April at the end of the winter period of hibernation and fasting, two fresh specimens were obtained for use as spring controls.

Blood was drawn by syringe from the aortæ and sinus venosus and allowed to clot in tubes. After centrifuging, the supernatant serum was removed by pipette. A clear, colorless fluid which was frequently found in considerable amounts in the body cavity was removed by syringe without contamination by blood. It is this fluid which is referred to as body fluid.

Colloid osmotic pressures were obtained by the second method of Krogh and Nakazawa (6), using the modifications suggested by Turner (7). The membrane was Du Pont cellophane no. 450, the outer liquid 0.6 per cent sodium chloride. For every determination three or more osmometers were set up. The figure taken for the colloid osmotic pressure of any sample was the average of values given by osmometers which conformed to the requirements of the Krogh technique. Determinations of the total and non-protein nitrogen concentrations were made by the micro-Kjeldahl method combined with direct Nesslerization as given by Peters and Van Slyke (8). The difference between total and non-protein nitrogen gave the concentration of protein nitrogen. Total refractive indices were determined by a Zeiss dipping refractometer. Whenever the quantity of serum available made it possible, a determination was made of the refractive index of an ultrafiltrate prepared by the use of a Thiessen ultrafiltration apparatus with cellophane 450 as membrane. The total refractive index minus that of the ultrafiltrate gives the refractive index of the colloid fraction.

Results

The protein data on sera and body fluids are given in Table I. The serum colloid osmotic pressure in animals of the warm, fed group was within the range of the normal fall values for a period of three months. The progressive decrease in pressure observed in all the other groups may be explained as due to the effects of starvation modified by the metabolic rates of the animals. In the warm, starved group total starvation plus a relatively high metabolic rate resulted in marked and immediate changes in the serum proteins as shown by the lowered colloid osmotic pressure. Edema was expected in these animals, but

was not found either in this or in any other group. Since the turtles in the cold, fed group ate very little, both cold groups may be regarded as starved. Due apparently to the depression of the metabolism by the low temperature, the colloid osmotic pressure in these animals decreased more slowly than in the warm, starved group and the total fall was less. That the rate of metabolism in animals when subjected to different environmental conditions is so affected was shown by

TABLE I

Protein data from blood serum and body fluid, *Malacoclemmys geographica*. Body fluid was found only in the animals indicated. Protein nitrogen was obtained by subtracting the analytically determined non-protein nitrogen from the total nitrogen. The refractive index of the colloid fraction was obtained by subtracting the refractive index of the ultrafiltrate from the refractive index of the whole serum. An asterisk after the colloid refractive index indicates that in this case no ultrafiltrate was available. The figure was obtained as stated in the text.

Status of animal	Date killed	Length of experimental period	Colloid osmotic pressure		Total nitrogen		Protein nitrogen		Refractive index		
			Serum	Body fluid	Serum	Body fluid	Serum	Body fluid	Serum	Colloid fraction	Body fluid
		months	*mm. H_2O*	*mm. H_2O*	*mgm./cc.*	*mgm./cc.*	*mgm./cc.*	*mgm./cc.*			
Normal, fall	Oct. 30	0.0	81	81	—	—	—	—	1.34350	.00768	1.33586
" "	Nov. 7	0.0	112	—	—	—	—	—	1.34453	.00886*	—
Warm, fed	Jan. 4	2.0	98	45	6.54	0.75	6.29	0.61	1.34364	.00799	1.33586
" "	Feb. 2	3.0	99	—	6.83	—	6.60	—	1.34399	.00809	—
Warm, starved	Dec. 21	1.75	58	—	6.24	—	6.02	—	1.34508	.00907	—
" "	Jan. 17	2.5	58	28	5.85	0.83	5.45	0.51	1.34291	.00758	1.33632
" "	Mar. 1	4.0	39	36	6.58	0.79	6.34	0.58	1.34342	.00779	1.33636
Cold, fed	Dec. 14	1.5	94	50	5.64	1.77	5.51	1.65	1.34341	.00773	1.33659
" "	Jan. 15	2.5	77	—	6.95	—	6.73	—	1.34474	.00907*	—
" "	Feb. 12	3.5	72	—	6.14	—	6.04	—	1.34339	.00766	—
Cold, starved	Dec. 11	1.3	102	65	5.37	0.66	5.19	0.54	1.34356	.00789*	1.33604
" "	Jan. 10	2.3	57	41	4.70	1.23	4.55	1.07	1.34291	.00724*	1.33519
" "	Jan. 24	2.7	58	34	6.51	1.23	6.27	1.00	1.34350	.00760	1.33580
" "	Feb. 7	3.25	66	—	5.74	—	5.56	—	1.34548	.00981*	—
Normal, spring	Apr. 7	0.0	68	—	4.18	—	4.02	—	1.34208	.00671	—
" "	Apr. 9	0.0	48	—	5.11	—	4.90	—	1.34235	.00699	—

Hand (9) in his compilation of the results of inanition on the higher groups of animals.

The occurrence of amounts of body fluid adequate for determinations was irregular and not limited to any group or groups nor to any particular time in the experimental period. Although the variations in the colloid osmotic pressure were wide, the value in each case was found to be lower than that of the corresponding serum except for one instance of equality. No proportional relation between the two pressures was discernible.

Table I shows total nitrogen and protein nitrogen figures, the latter obtained by subtracting the non-protein nitrogen from the total nitrogen. The non-protein nitrogen content was low and varied irregularly in the sera from .10 to .40 mgm./cc., with eleven of the fourteen values between .15 and .25 mgm./cc. In the seven analyses of body fluid the variation of non-protein nitrogen was from .12 to .32

TABLE II

A table showing *percentage* changes as the experiment progressed. The two normal fall animals are taken as 100 per cent. In the case of the nitrogen values, since no analyses were made on the fall animals, the 100 per cent is furnished by the two warm, fed animals whose other findings showed a negligible decrease as the months went by. The two spring animals were fresh from the collector, after a hibernation period of unknown duration probably much longer than the duration of the experiment.

Status of animal	Length of experimental period	Colloid osmotic pressure	Protein nitrogen	Refractive index
	months	*per cent*	*per cent*	*per cent*
Normal, fall	0.0	100	—	100
Warm, fed	2.0	101.5	100	96.5
" "	3.0	102.5	100	98
Warm, starved	1.75	60	93.5	110
" "	2.5	60	84.5	92
" "	4.0	40.5	98	94
Cold, fed	1.5	97.5	85.5	93.5
" "	2.5	80	104	110
" "	3.5	75	94	93
Cold, starved	1.3	106	80.5	95.5
" "	2.3	59	70.5	87.5
" "	2.7	60	97	92
" "	3.25	68.5	86	118
Normal, spring	—	70.5	62	81
" "	—	50	75.5	84.5

mgm./cc. The concentration of protein varied widely yet it seemed clear that starvation in this species fails to cause a fall in the amount of protein parallel to the fall in colloid osmotic pressure. Table II gives the values in percentages, thus showing the lack of correspondence between the effect of starvation on colloid osmotic pressure and on protein content. The average of the two fall specimens is taken as 100 per cent for the colloid osmotic pressure series. It will be noted that the warm, fed animals show no fall. Nitrogen concentrations

were not determined for the two fall animals but in the absence of that standard it seemed permissible to use the level of the two warm, fed animals as 100 per cent for protein comparisons. Determinations of total protein, albumin, and globulin in the sera of mammals have been reported by several observers (10, 11, 12, 13, 14) who have shown that during starvation a reversal of the mammalian albumin-globulin ratio occurs, indicative of a relatively higher globulin concentration. Because of the larger size of the globulin molecules this shift results in a marked fall in colloid osmotic pressure without a corresponding decrease in total protein concentration. From our observations it seems possible that a similar shift in albumin-globulin relations may occur in the turtle. The two spring specimens which had doubtless spent the entire winter in hibernation showed a fall in total protein more nearly approaching that in colloid osmotic pressure. The concentration of protein in the body fluid was much lower than in the serum and showed wide variations. No direct correlation with the colloid osmotic pressure was apparent.

The total refractive index of normal, fall turtles averaged 1.34377. With two exceptions the figures for animals with lowered colloid osmotic pressure were below this average, falling even to 1.34222 as an average for the two spring controls. The range of the refractive indices for the ultrafiltrates was narrow, with an average of 1.33569. Where no ultrafiltrate could be prepared, this average figure was used to calculate the probable value for the colloid fraction. (See Table I.) When the refractive index of the colloid fractions was plotted against the colloid osmotic pressure a good correspondence was evident, though there is a closer correspondence between protein concentration and refractive index of colloid fractions. The refractive index of the body fluid was consistently lower than that for the corresponding serum and higher than that of the serum ultrafiltrate. Since the crystalloids of serum and body fluid are probably closely alike, this difference between body fluid and ultrafiltrate from serum would indicate a colloid fraction in the body fluid, as verified by analysis.

Discussion

Our interest has centered upon the inferences which may be drawn from these determinations as to the significance of the serum proteins. Certain points stand out: first, the fall in colloid osmotic pressure with prolonged starvation, especially at the higher temperature; second, the much greater stability shown during starvation in the quantity of serum protein as compared with its colloid osmotic pressure; third, the presence in the body fluid of substances exerting a colloid osmotic

pressure far from negligible though the protein percentage is low. While the relation of plasma and true tissue fluid—meaning by this the fluid which has just made its exit through capillary walls—may not be that shown by the comparison of the serum and body fluid, it is nevertheless probable that capillary permeability to proteins, at least those of the smaller molecular size, is indicated by the very perceptible colloid osmotic pressure of the body fluid. Such proteins as may be outside capillary walls will, of course, lessen the effective colloid osmotic pressure of the plasma. Further, the marked decline of the colloid osmotic pressure in starvation without evident edema speaks against it as a major controlling factor in water balance between blood and tissues in this species, while the persistence of the protein content of the serum at a relatively high level may indicate the importance of plasma proteins for some other function than the maintenance of colloid osmotic pressure, possibly as nutritional reserves.

Summary

The average serum colloid osmotic pressure of two normal fall turtles of the species *Malacoclemmys geographica*, Les. was 96 mm. water pressure. This pressure was lowered by starvation, especially at laboratory as compared with winter temperature, but the serum protein concentration was much less affected. A body fluid obtainable from about half of the animals showed a colloid osmotic pressure of more than half the serum pressure in these individuals, though the protein content was low. These findings are interpreted as indicating that in this species the colloid osmotic pressure may not be the only controlling factor in the water balance between blood and tissues, and that other functions of plasma proteins may be of importance.

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SERUM PROTEIN MEASUREMENTS IN THE LOWER VERTEBRATES. II. IN MARINE TELEOSTS AND ELASMOBRANCHS

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The reasons for studying the plasma proteins of the lower vertebrates as shown in the first paper of this series (1) have to do with the validity of Starling's hypothesis for these forms and with the possible relation of the protein fraction of the plasma to nutritional needs. References to contributions in this field relating to the higher vertebrates will not be repeated. For the lower vertebrates, the list given by Drinker and Field (2) summarizes the information available up to their date of publication. A brief paper by Keys and Hill (3) gives the only findings for the colloid osmotic pressure of fish sera except those in two preliminary notes to this paper (4, 5). Interesting data allied in various ways are to be found in references (6) to (15) inclusive. The last two papers (14, 15) have excellent bibliographies.

The serum of various species of marine teleosts and elasmobranchs was studied at the Woods Hole Oceanographic Institution during the summers of 1933 and 1934, and during the summers of 1935 and 1936 at the Bergens Museum Biologiske Stasjon on the island of Herdla off the west coast of Norway. To both of these institutions most sincere thanks are due for the assistance generously rendered by them. A grant in 1936 from the Tracy McGregor Fund helped to defray expenses in that year.[2]

The determinations made were as follows: (1) colloid osmotic pressure, on all samples; (2) refractive index of the whole serum, on all samples; (3) refractive index on an ultrafiltrate, on many samples of the Norwegian series, whenever quantity of serum permitted; (4) total nitrogen and non-protein nitrogen in 1934, 1935, and 1936, a con-

[1] Contribution No. 148.

[2] The nitrogen determinations of the Woods Hole series were made by Catherine Goffin and those of the Norwegian series by Cand. mag. Francis Wolff, of Bergens Museum. Acknowledgment is made of the careful and helpful work done by both. At Woods Hole the Marine Biological Laboratory made its facilities available to Miss Goffin for her work; at Bergen the Norwegian Fiskeriforsöksstasjon allowed the use of its equipment for cold storage and for chemical work. Without this help it would have been impossible to make the nitrogen analyses.

siderable number of determinations, whenever quantity permitted. In all, 121 individual samples representing 19 species of teleosts were studied and 48 individual samples from 11 species of elasmobranchs. A few of these samples were assembled from two or more small fish each. There were in addition some scattered determinations on other species.

Methods

Obtaining the Fish

The fish whose blood is to be studied should be in a healthy condition and must be uninjured, to prevent access of the surrounding medium to the blood. To secure fish that meet these standards is difficult. The ordinary methods of fishing are extremely rough when viewed from the standpoint of obtaining true samples of the circulating blood. The effect of the severe struggling often incident to capture may not be negligible. Grafflin (8) has told in detail of his care in this matter of reliable sampling. The fish included in this study have been progressively better and none are included in the figures reported about which there is appreciable doubt. Two alternatives present themselves. Blood may be drawn the moment the fish is taken and this is for some species, for example those from deep water which will not live in tanks, the only possible method; or the fish may be allowed to rest for some hours or longer in cars or tanks of water suitable in temperature and aeration. Feeding is possible in some cases where the fish live well in captivity. The effect of the initial struggling is thus eliminated and the state of the fish is steadier. At least four of the most consistent sets of determinations in this study have been made mainly on fish thus treated, *Opsanus tau*, *Tautoga onitis*, *Anarhichas lupus*, and *Raia erinacea*. In certain species the skin is so delicate that it is probably best not to use them in such studies as these unless unusual care can be taken. It is possible that the two pleuronectids in the Woods Hole series belong in this group.

Enough is known about the relation existing between the blood and tissues of fishes and the demands of the breeding season to make avoidance of this period desirable in a preliminary survey. However, the length of this season is often far beyond the months mentioned in books so that it has not always been possible to avoid it without sacrificing the use of fish otherwise very suitable. The depression in the breeding season well-known to trout hatchery men, when the fish will eat nothing or very little, is of indeterminate length and not easily avoided. The breeding fish used in this series are so described. When it is not mentioned that a species was apparently ripe it is to be understood that as far as known the samples were not from breeding fish.

Blood Sampling

The blood was taken in 1933 by syringe from gill vessels or heart; in the other three summers, in most cases from tail vessels, occasionally from the heart. For the suggestion of taking blood from the tail, the author is indebted to Dr. Homer W. Smith. The fish is wrapped in a cloth, laid in a deeply grooved board, ventral side up, restrained by lacing tapes across the board, and held by an assistant. The syringe needle is inserted in the mid-ventral line, somewhat behind the anus, often along the anal fin, at an angle which will take it between the hæmal spines. It is pushed in until the vertebral column is felt, is slightly withdrawn and pushed in again quickly, with a good chance of puncturing a tail vessel from which blood can be withdrawn with a minimum of injury and a maximum of ease. If the tail vessels are too small for use or are covered by too firm a membrane between the spines, the heart is exposed, the ventral aorta clamped off by a hemostat, and blood withdrawn from the bulbus or venous sinuses. The blood must be handled gently when discharged from the syringe into the centrifuge tubes else hemolysis may occur. The same thing may happen if the vessel used is not entered quickly and neatly. Heparin has been used in both syringe and centrifuge tubes, but some clotting is usually evident after centrifuging, hence the term serum has been thought more appropriate than plasma.

The blood was centrifuged three or four hours after being withdrawn, not earlier because in that case delayed and repeated clotting sometimes occurs and is very troublesome if in osmometer tubes. After centrifuging, the serum was set up at once in the osmometers or kept in a very cold refrigerator until next day. Because it was impossible in Norway to have the nitrogen determinations made on the first day, various preservatives were tried, but all resulted in the appearance of a precipitate. Serum kept at about 0° C., on the other hand, remains clear for a period up to at least three weeks, and refractometric measurements show little or no alteration. At rather low room temperatures, 16°–19° C., osmometers containing teleost serum have held steady as long as three days. In the case of elasmobranch blood, the occasional rather warm intervals at Woods Hole, with room temperatures up to 25°, in a few instances caused the appearance of a thin whitish film on the membrane in the osmometer, but the colloid osmotic pressure in these cases seldom varied from the usual range. These films were not seen at the temperature of the Herdla laboratory, usually 15°–18° C.

A sterile technique was not attempted. The place where the needle was to be pushed through the skin was carefully wiped with

cotton. Syringes, needles, centrifuge and transfer tubes, and all other parts of apparatus coming into contact with the serum were cleaned systematically as follows. They were washed thoroughly with water, with soap when needed; they were rinsed thoroughly first with tap water, then with distilled water, finally with 70 per cent alcohol; they were dried in an oven at 70°–80° C. Contamination was not apparent except in the elasmobranch samples of the Woods Hole series in the hottest weather.

In general, then, while the securing, care, and preservation of the blood have presented difficulties, the samples included in this report

TABLE I

Protein determinations on serum of *Opsanus tau*, toadfish. Fish all of one lot, kept in aquarium up to 10 days, fed. One or two months after the breeding season. The length of the specimens varied from ten to twelve inches. Nos. 1 and 6 were females, the others males. C.O.P. Colloid osmotic pressure in mm. water pressure, determined from one or two osmometers. N-P. N. Non-protein nitrogen.

No.	Av. C.O.P.	Refractive index	Total N.	N-P. N.	Protein N.
	mm./water		*mg./cc.*	*mg./cc.*	*mg./cc.*
1	104	1.34401	—	—	—
2	119	1.34498	8.13	.866	7.26
3	76	1.34498	9.28	.809	8.47
4	100	1.34470	6.73	.494	6.24
5	95	1.34375	6.77	.497	6.27
6	127	1.34369	6.55	.419	6.13
7	125	1.34457	5.63	.423	5.21
8	111	1.34386	7.44	.368	7.07
9	97	1.34509	—	—	—
10	84	1.34468	—	—	—
Averages	*104*	*1.34443*	*7.22*	*.554*	*6.67*

are considered reasonably good. However, it is to be said that the history of the individual fish is in many important points entirely unknown. Its age, except as indicated roughly by its size, its recent activity when the fish has been bled immediately on being taken, its nutritive condition except that it may or may not have food in the digestive tract, all these factors of importance are not known. A considerable range in findings is to be expected.

The Identification of Species

The identification of species was made at Woods Hole by the use of Bigelow and Welsh (16) and in Norway by Otterström (17). In both places much help was received from various persons who know the

fishes of the region. Especial mention should be made of the Director at the Herdla laboratory, Professor August Brinkmann, and his son, and of the Assistant-Director, Dr. Rustad.

Methods of Testing

Colloid osmotic pressures were determined by the method of Krogh and Nakazawa (18) somewhat modified (19). The membrane used was cellophane no. 450. Refractive indices were determined by the dipping refractometers of Bausch and Lomb and Zeiss on both whole serum and its ultrafiltrate. The latter was secured from samples of

TABLE II

Protein determinations on serum of *Anarhichas lupus*, wolf-fish, 1935. Blood taken a few hours or a day after fish was brought to laboratory. Not near the breeding season. The length of the specimens varied from 18 to 30 inches. C.O.P. Colloid osmotic pressure in mm. water pressure. Derived from two to four osmometers in all cases except where only one osmometer was used, Fish No. 7. N-P. N. Non-protein nitrogen.

No.	Av. C.O.P.	n_D Serum	n_D Ultrafiltrate	n_D Colloid fraction	Total N.	N-P. N.	Prot. N.
	mm./water				*mg./cc.*	*mg./cc.*	*mg./cc.*
1	119	1.34530	1.33532	0.00998	6.15	.25	5.90
2	178	1.35588	1.33609	0.01979	10.94	.51	10.43
3	147	1.35051	1.33609	0.01442	7.96	.52	7.44
4	152	1.35128	1.33636	0.01492	8.55	.62	7.93
5	145	1.34702	1.33545	0.01157	8.12	.71	7.41
6	153	1.34832	1.33551	0.01281	—	—	—
7	150	1.34415	1.33551	0.00864	5.82	.77	5.05
8	177	1.34984	1.33578	0.01406	8.30	.77	7.53
Averages	*153*	*1.34916*	*1.33576*	*0.01328*	*7.98*	*.69*	*7.38*

the Norwegian series by a Thiessen apparatus, with care as to uniformity of technique. Two cubic centimeters of serum were placed in the apparatus with cellophane no. 450 as membrane and subjected to a pressure of some three atmospheres for a period of 12 hours. At the end of this time a clear, limpid filtrate was obtained, always protein-free. The residue on the membrane at this time was small in amount and slimy in character. Tests for chloride and reducing sugar showed them to be present in the ultrafiltrate. It was assumed that the membrane was permeable to practically all the serum crystalloids.

Nitrogen determinations were made in 1934 at Woods Hole by the micro-Kjeldahl method with direct Nesslerization. For the non-protein determinations the precipitant was trichloracetic acid. In

1935 and 1936 the micro-Kjeldahl method was by titration. The precipitant was metaphosphoric acid. All methods and reagents were suitably controlled and tested.

TABLE III

The colloid osmotic pressure of the sera of marine teleosts. Arranged in order of height of osmotic pressure, in two series.

Name of species	Number of individuals	C.O.P. Range	C.O.P. Average
		mm. water	*mm. water*
Woods Hole Series			
Sarda sarda, Bloch. Bonito	1	233	233
Echeneis naucrates, Lin. Remora*	1	216	216
Tautoga onitis, Lin. Tautog*	24	79–193	129
Paralichthys dentatus, Lin. Summer flounder	6	79–180	120
Pseudopleuronectes Americanus, Wal. Winter flounder	7	86–122	106
Prionotus Carolinus, Lin. Common robin*	7	87–117	102
Prionotus strigatus, Cuv. & Val. Red-winged robin*	10	76–127	104
Norwegian Series			
Scomber scombrus, Lin. Mackerel	3 samples fr. 11 fish‡	164–212	198
Anguilla vulgaris, Tur. Eel	4 samples fr. 13 fish‡	167–204	189
Belone acus, Risso. Hornfish	2	162, 184	173
Anarhichas lupus, Lin. Wolf-fish	13	85–181	147
Brosmius brosme, Asc. Cusk†	8	105–210	139
Molva molva, Flem.†	4	108–177	139
Gadus morrhua, Lin. Cod	11	70–192	117
Gadus pollachius, Lin. Pollack	8 samples 3 fr. 6 fish‡	31–172	94
Cyclopterus lumpus, Lin. Lumpfish*	3	75–87	81
Lophius piscatorius, Lin. Goosefish	8	25–60	42
Gadus virens, Lin.	1 fr. 2 fish‡	40	40

* Breeding season.

† From deep trawl, 300–400 meters. Fish with swim-bladders protruding or burst and with organs extruded in some cases on arrival at surface. Blood samples taken immediately on board the collecting boat.

‡ Samples from more than one fish because of small size of samples available from single individuals. The refractive indices were found to be similar before samples were combined.

RESULTS

The results of the study are presented in a series of annotated tables. The determinations on teleosts are given first. To show the range of variation among individuals of a single species *Opsanus tau*, Lin., toadfish, was chosen from the Woods Hole series of 1934, Table I,

and *Anarhichas lupus*, Lin., wolf-fish, Table II, from the Herdla series of 1935. The toadfish was chosen as a sluggish fish, much studied at Woods Hole laboratories, and the wolf-fish as a more vigorous species much studied in Norway.

TABLE IV

Refractive indices of sera and ultrafiltrates from marine teleosts. Arranged in order of height of C.O.P. Figures in parentheses indicate the number of individuals used for the following average, when this is less than the total number.

Name of species	Number of individuals	n_D Serum	n_D Ultrafiltrate	n_D Colloid
Woods Hole Series				
Sarda sarda	1	1.35220	—	—
Echeneis naucrates	2	1.35158	—	—
Tautoga onitis	24	1.34629	—	—
Paralichthys dentatus				
Pseudopleuronectes Americanus	6	1.34313	—	—
Prionotus Carolinus	7	1.34329	—	—
Prionotus strigatus	7	1.34205	—	—
Opsanus tau	10	1.34443	—	—
Norwegian Series				
Scomber scombrus	3 samples fr. 11 fish*	1.35000	(2)1.33690	0.01306
Anguilla vulgaris	4 samples fr. 13 fish*	1.35152	1.33552	0.01600
Belone acus	2	1.35084	1.33724†	0.01360
Anarhichas lupus	13	1.34852	1.33577	0.01275
Brosmius brosme	5	1.34703	1.33574	0.01169
Molva molva	4	1.34597	(2)1.33601	0.00912
Gadus morrhua	11	1.34421	1.33588	0.00834
Gadus pollachius	8 3 of these fr. 6 fish*	1.34401	1.33594	0.00807
Cyclopterus lumpus	3	1.34388	1.33535	0.00853
Lophius piscatorius	8	1.34168	1.33545	0.00623
Gadus virens	1 sample fr. 2 fish*	1.34158	1.33559	0.00599

* See note on Table III.

† Samples used for obtaining ultrafiltrates in these cases were only one cubic centimeter instead of two as usual. This may explain the aberrant value.

Table III summarizes the data on colloid osmotic pressure from the teleosts of Woods Hole and Herdla; Table IV, the data on refractive indices; Table V, the nitrogen determinations. The fish from Woods Hole and Herdla are listed separately, since the procedures were more complete in the later series. The order of names in all tables is that of the relative heights of the colloid osmotic pressure, even though the

order of refractive indices and of nitrogen content does not follow exactly the same course.

In a similar manner Tables VI–IX present the data for elasmobranchs, Table VI showing the variation among individuals of a single species, *Raia erinacea*, Lin., small skate, and Tables VII–IX the colloid osmotic pressure, refractive indices, and nitrogen findings respectively for the several species of elasmobranchs.

An effort was made to study *Myxine glutinosa*, Lin., hagfish, but it

TABLE V

Nitrogen determinations on sera of marine teleosts.

Name of species	Number of individuals	Total N.	N-P. N.	Protein N.
		mg./cc.	*mg./cc.*	*mg./cc.*
Woods Hole Series				
Echeneis naucrates	1	6.68	.272	6.41
Tautoga onitis	6	6.62	.498	6.12
Paralichthys dentatus				
Pseudopleuronectes Americanus	3	5.48	.452	5.03
Prionotus Carolinus	1	7.90	.747	7.15
Opsanus tau	7	7.22	.554	6.67
Norwegian Series				
Scomber scombrus	2 samples fr. 9 fish*	8.30	.65	7.66
Anguilla vulgaris	3 samples fr. 11 fish*	8.47	1.19	7.28
Anarhichas lupus	10	8.11	.70	7.48
Brosmius brosme	1	6.95	.57	6.38
Gadus morrhua	5	5.93	.60	5.33
Gadus pollachius	3 samples fr. 5 fish*	6.11	.76	5.34
Cyclopterus lumpus	3	5.37	.25	5.09
Lophius piscatorius	8	4.85	.44	4.41
Gadus virens	1 sample fr. 2 fish*	3.99	.39	3.60

* See Table III.

was found very difficult to secure blood samples entirely uncontaminated by slime. The individuals were not large enough to yield more than about one cubic centimeter of blood, often less, and therefore of necessity the determinations were made on mixed samples, though the refractive indices were taken before mixing and only samples of similar indices combined. From one such mixed sample two osmometers gave an average of 24 mm. c.o.p., the total serum showed a n_D of 1.35073, the ultrafiltrate n_D 1.33923, thus giving a colloid index of 0.01150. The total N of the serum was 5.88 mgm./cc.

TABLE VI

Protein determinations on serum of elasmobranch, *Raia erinacea*, small skate. Fish of different lots, kept in aquarium not more than three or four days. Height of breeding season, several eggs laid in aquarium. The length of the specimens varied from 17 to 19 inches. Nos. 2, 3 and 9 were males, the rest females.

C.O.P. Colloid osmotic pressure, determined from one to three osmometers, usually two.

N-P. N. Non-protein nitrogen.

No.	Av. C.O.P.	Refractive index	Total N.	N-P. N.	Protein N.
	mm./water		*mg./cc.*	*mg./cc.*	*mg./cc.*
1	63	1.34694	16.83	—	—
2	73	1.34886	16.95	—	—
3	14	1.34665	—	—	—
4	50	1.34581	—	—	—
5	47	1.34895	—	—	—
6	71	1.34765	—	—	—
7	17	1.34661	15.91	—	—
8	16	1.34661	14.78	13.55	1.23
9	14	1.34849	13.13	13.02	0.11
10	34	1.34683	16.39	13.19	3.20
11	19	1.34618	16.39	13.71	2.68
12	29	1.34640	16.75	13.88	2.87
Averages	*37*	*1.34715*	*15.49*†	*13.47*†	*2.02*†

† Five only used for average.

TABLE VII

The colloid osmotic pressure of the sera of elasmobranchs, arranged in the order of the height of the osmotic pressures.

C.O.P. Colloid osmotic pressure.

Name of species	Number of individuals	C.O.P. Range	C.O.P. Average
		mm./water	*mm./water*
Woods Hole Series			
Carcharias taurus, Raf. (sp.?) Shark	1	46	46
Mustelus canis, Mitch.† Smooth Dogfish	4	33–58	41
Raia erinacea, Mitch.† Small Skate	12	14–73	37
Dasybatus marinus, Klein. Stingaree	1	36	36
Norwegian Series			
Raia fullonica, Lin.‡	2	14, 49	32
Chimæra monstrosa, Lin.‡	5	10–40	27
Galeus vulgaris, Flem. Shark	1	27	27
Squalus acanthias, Lin.‡ Spiny Dogfish	2	23, 31	27
Pristiurus catulus, Gun.‡	7	5–59	26
Raia oxyrhynchus, Lin.† ‡	6	11–45	22
Etmopteris spinax, Lin.† ‡	7 samples fr. 14 fish*	–8–34	17

* Individual fish small, about 12–14 inches. Samples were therefore combined, but only after refractive indices had shown them to be in the same range. Two negative values may indicate the error of the method when the pressure is extremely low.

† Breeding season.

‡ From deep trawl, 300–400 meters. No outward sign of injury on coming to the surface. *E. spinax* and *R. fullonica* lived often as long as 24 hours in laboratory tanks.

TABLE VIII

Refractive indices of sera and ultrafiltrates from elasmobranchs. The number in parentheses (4) indicates that the following n_D was derived from 4 individuals only. See notes on Table VII.

Name of species	Number of individuals	n_D Serum	n_D Ultrafiltrate	n_D Colloid
Woods Hole Series				
Carcharias taurus	1	1.34601	—	—
Mustelus canis†	4	1.34618	—	—
Raia erinacea†	12	1.34715	—	—
Dasybatus marinus	1	1.34682	—	—
Norwegian Series				
Raia fullonica‡	2	1.34852	1.34079	0.00774
Chimæra monstrosa‡	5	1.34596	(4) 1.34063	0.00486
Galeus vulgaris	1	1.34422	1.34113	0.00309
Squalus acanthias‡	1	1.34671	1.34096	0.00575
Pristiurus catulus‡	7	1.34423	1.34076	0.00331
Raia oxyrhynchus† ‡	5	1.34412	1.34070	0.00342
Etmopteris spinax† ‡	7 samples fr. 14 fish*	1.34353	1.34055	0.00288

the N-P.N., 0.22 mgm./cc. These figures do not seem concordant, but are reported since data on this form are few.

Body fluid was present in one instance only in quantity sufficient for collection and study. This was in a wolf-fish of medium size and

TABLE IX

Nitrogen determinations on sera of elasmobranchs. See notes on Table VII. Number in parentheses (1) indicates that in only one instance was there serum enough for the determination of the N-P. N.

Name of species	Number of individuals	Total N	N-P. N.	Protein N.
		mg./cc.	*mg./cc.*	*mg./cc.*
Woods Hole Series				
Raia erinacea†	5	15.49	13.47	2.02
Dasybatus marinus	1	16.85	11.81	5.04
Norwegian Series				
Raia fullonica‡	3	17.50	12.16	5.34
Chimæra monstrosa‡	1	13.91	11.15	2.76
Galeus vulgaris	1	13.76	11.47	2.29
Squalus acanthias‡	1	14.98	12.68	2.30
Pristiurus catulus‡	3	12.72	9.58	3.14
Raia oxyrhynchus† ‡	6	15.07	12.31	2.76
Etmopteris spinax† ‡	4 samples fr. 5 fish*	12.18	(1) 10.07	1.60

medium serum values. There was nothing to indicate that the fish was not in a healthy condition. The data are given in Table X. The body fluid showed a c.o.p. nearly four-fifths that of the serum, and while the refractive index did not show quite as high a colloid fraction, yet it is clear that this fluid contained a considerable amount of protein, an amount comparable to that found in the body fluid of the "slider" turtle (1).

Discussion of Results

Regarding the results on teleosts, it is apparent that the findings for a single species show a range proportionately wider that we expect in mammals. Such a lack of constancy has been found by other workers on fish blood. (See references in the first paragraph of this paper.) Whether this is really true or due, in spite of efforts to the contrary, to the use of unsuitable specimens is not known. Studies are in progress on fresh-water species living in the controlled environ-

Table X

Comparison of serum and body fluid from one specimen of *Anarhichas lupus* from which enough body fluid was obtainable for colloid osmotic pressure and refractometer tests though not for nitrogen analyses.

Fluid	C.O.P.	n_D Serum or fluid	n_D Ultrafiltrate	n_D Colloid
	mm./water			
Blood serum	129	1.34707	1.33563	0.01144
Body fluid	98	1.34184	1.33532	0.00652

ment of fish hatchery ponds to see if the variation from individual to individual is as great as that seen in the toadfish, 76–127 mm. or in the wolf-fish, 119–178 mm. water pressure. Also the yearly rhythm is being followed to see whether colloid osmotic pressure shows the effect of elaboration of sex products and of spawning depression.

There is obviously a wide difference between the colloid osmotic pressure of the goosefish, 42 mm. and that of the mackerel, 198 mm. This latter value is similar to that found by Keys and Hill (3) for *Anguilla*, the European eel, and verified in this series. The ranges for individuals of these species are seen to be mutually exclusive, but those for species nearer the middle of the total range overlap freely and some are indistinguishable, as for instance the two species of *Prionotus*. To what this characteristic range for a species is related is not known though various suggestions have been made. Arterial pressure determinations on fish are few and reliable determinations still fewer (20), while determinations of capillary pressure have not been

made at all. The degree of habitual activity has been suggested as a clue, for *Lophius* is proverbially sluggish and *Scomber* very active, while the extensive migrations of *Anguilla* are well-known. The series is as

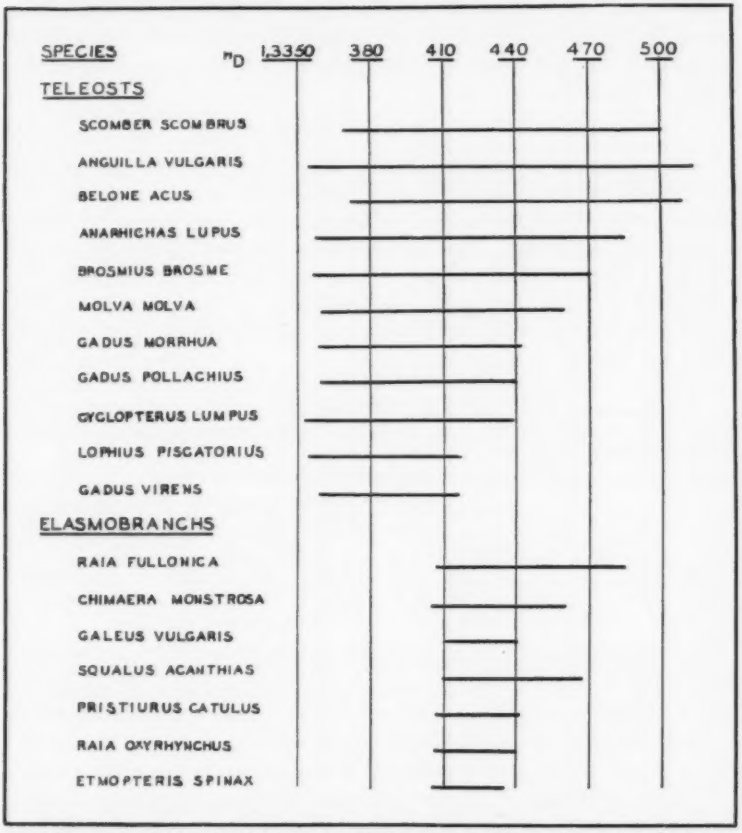

CHART 1. Refractive indices of colloid fraction in Norwegian series of teleosts and elasmobranchs. The left end of each line shows the refractive index of the ultrafiltrate, the right end that of the whole serum. The length of the line therefore shows the index of the colloid fraction. The ultrafiltrates in all of the teleosts except two are closely grouped, indicating relative constancy in the non-colloid components of the sera. The two exceptions are in fish which were represented by limited samples only, in the case of Belone by samples for ultrafiltration of only half the usual size. The high position of the ultrafiltrate index for all elasmobranchs is related to their high non-protein nitrogen fraction.

yet too short to give an answer here. The possibility of other substances which may add to the colloid osmotic pressure of the serum, e.g. fats (21, 22) is not to be excluded particularly when one occasion-

ally sees globules of fat at the top of a centrifuged tube of blood and infers the presence of associated substances in solution in appreciable amounts. Also the wide variation in the fat content of fish is a matter of common knowledge. No determinations on serum fats have been made. The albumin-globulin ratios in fish are said by Lepkovsky

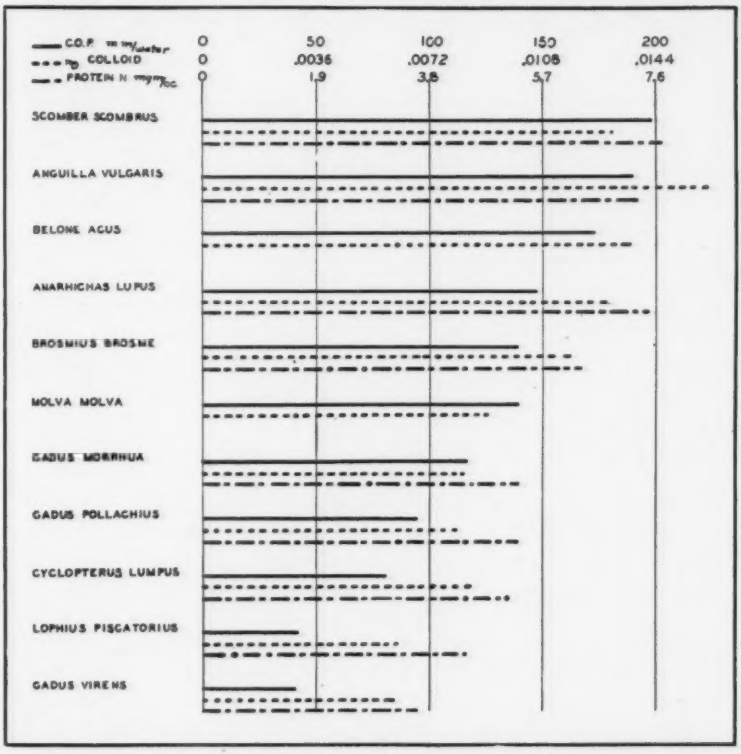

CHART 2. Comparison of colloid osmotic pressure, refractive index of colloid fraction, and protein nitrogen in Norwegian teleost series. The several ranges have been adjusted so that the maximum values are shown by lines of approximately equal length. The relative values of the three measurements thus appear, with a range much wider in c.o.p. than in n_D or protein N. This is probably to be explained by a variation in the albumin-globulin ratio in the direction of large molecules when the total quantity is small.

(11) to vary widely. Such variations, with their attendant contrast in the size of the protein molecules, may help to explain not only differences between species but irregularities in the relationships between colloid osmotic pressure and other protein data. A few preliminary studies on plasma protein regeneration after depletion by

hemorrhage seem to indicate that protein may come back rather quickly, perhaps more quickly in total quantity than in colloid osmotic pressure, which may be the same as saying that the globulins regenerate—or are gotten into the blood—more rapidly than the albumins. This obviously suggests the experiments on mammalian protein regeneration from the laboratories of Whipple and Weech, to whose papers only partial references are given (23, 24, 25).

As the refractive indices are scrutinized, it is apparent that the range for the ultrafiltrates is narrow, indicating constancy in the non-protein fraction of the plasma, while the refractive index for the colloid fraction varies widely from species to species and accompanies the c.o.p. though the correspondence is not too close. (See Chart 1.) The analyses for total and non-protein nitrogen similarly give values for the serum proteins which vary approximately with the c.o.p. but follow more closely the refractive indices of the colloid fractions. These correspondences and differences are brought out in Chart 2. It is to be noted that the range of c.o.p. from the species of low to those of high values is much greater than the range in protein content. This fact may be associated with the ready appearance of protein molecules of large size mentioned above. It is possible that in the life economy of teleosts it is of especial importance to hold the total protein content of the serum at a definite level while the assortment of proteins may vary. It would follow from this that a rigid maintenance of colloid osmotic pressure is impossible or unnecessary.

For the elasmobranchs it is to be said that all averages for colloid osmotic pressure are low, though occasional individuals have been found as high as 70 mm. water pressure. As expected, the total and non-protein nitrogen findings, the refractive indices for total sera and for ultrafiltrates are all high, reflecting the high urea or trimethylamine oxide content of elasmobranch blood. The protein nitrogen figures are low with two exceptions, one specimen of *Dasybatus marinus*, stingray, and three of *Raia fullonica*, a Norwegian skate. Differences in the physiological make-up of elasmobranchs and teleosts thus include the protein blood picture.

Summary and Conclusions

1. In a study of 121 individuals of 19 species of marine teleosts it has been found that the range of colloid osmotic pressure shown by the blood serum is wide for a single species; that the c.o.p. shows a characteristic range in each species; and that for the species studied the c.o.p. varies from an average of 42 mm. water pressure in *Lophius piscatorius*, Lin., goosefish, to about 200 mm. in *Scomber scombrus*,

Lin., mackerel, *Anguilla vulgaris*, Tur., eel, and probably others. Intermediate values for averages within species were found most commonly, from about 100 to 150 mm.

2. The refractive indices for the colloid fractions of the sera and the protein nitrogen values follow the same general distribution as the c.o.p. though correspondence is not perfect. The c.o.p. seems to vary through a much wider range than the protein N or the refractive index of the colloid fraction.

3. All protein values for the 48 individuals of 11 species of elasmobranchs were found to be low, distinctly lower than in all save the very lowest of the teleosts.

4. The refractive indices of the ultrafiltrates from the sera in all teleosts were very constant as compared with the wide range of the colloid figures. This constancy, reflecting the crystalloid status of the sera, is also characteristic of the elasmobranch ultrafiltrates though the absolute level is materially higher in the latter group, a fact associated obviously with the high non-protein nitrogen content of elasmobranch blood.

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GENETICS AND HISTOLOGY OF THE COLOR PATTERN IN THE NORMAL AND ALBINO PARADISE FISH, MACROPODUS OPERCULARIS L.[1]

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The paradise fish is well known to fish fanciers. It is thought to have been the first "fancy" tropical fish to be bred in Europe, having been first introduced in Paris by Carbonier in 1868. Specimens of the albino variety utilized in this investigation were obtained from dealers in New York City during the fall of 1934. These were at that time a novelty and it is stated by Innes (1935) that they were first imported from German fanciers in 1933.

The paradise fish is one of the labyrinth fishes and is referred to Order Labyrinthici, Family (368) Osphronemidæ in the classification by Jordan (1923). It is also known in the literature as *Macropodus viridi-auratus*. A description is given by Regan (1909). This fish is found in the lowland streams of China, Formosa and Cochin China. It is well fitted to live in stagnant waters because its accessory respiratory apparatus, the labyrinth organ, allows it to use atmospheric oxygen as well as to breathe by gills. On this account almost no attention needs to be given to aeration of water in an aquarium and it may be kept in very small containers.

The paradise fish is a "bubble nest" builder. The male, presumably by aid of some oral secretion, makes a floating structure of bubbles into which he shoots the eggs as they are laid by the female. The mating and spawning activities, in our experience, take place at temperatures ranging from 26° C. to 29° C. Certain fish dealers, however, state that they will breed at as low a temperature as 20° C. Studies made by Goodrich and Taylor (1934) on a species *Betta splendens*, a related genus, showed a remarkably precise limitation of the breeding activities to a temperature of about 26° C. It is necessary, under aquarium conditions, to remove the female after laying, as she may eat the eggs, and to remove the male after hatching as he may eat the young fish. The young fry are first fed from Paramecia cultures and later with *Daphnia*. The adults are fed with enchytraeids

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(the white worms of dealers), minced earthworms, liver and dry prepared fish foods. Further details of the care of the fish are given by Innes (1935). The albino variety is much more difficult to rear. It is less viable and takes food less readily. It seems to avoid light more than the dark variety and possibly for this reason it is less likely to discover food.

Genetic Experiments

The dark or normal colored paradise fish when crossed with the albino variety produced all dark type in the F_1 generation. The F_2 in two separate crosses gave the results shown in Table I. The results

Table I

F_2 from cross between normal and albino varieties

Date of spawning	Number of dark	Number of albino	Ratio
9-18-35	204	62	3.29/1
1-21-36	438	145	3.02/1

F_1 from cross between heterozygous dark and albino

Date of spawning	Number of dark	Number of albino	Ratio
9-27-35	226	221	1.02/1
3-12-36	552	551	1.002/1

Counts in both cases made eight days after spawning.

of the back cross between the F_1 and the albino are also shown in Table I. Similar results were independently obtained by Kosswig (1935) though with somewhat greater deviation from the expected ratios. Our counts were made eight days after spawning and before the onset of the high mortality which is unfortunately frequent in fish cultures. These results clearly indicate that the normal and albino types are a pair of mendelian allelomorphs in which the normal is dominant over the albino.

The Color Pattern

The normal or wild type paradise fish (Fig. 1) is strikingly marked on the sides with vertical stripes which are referred to in this paper as green and red stripes. The color, however, varies with environmental conditions. The striping may largely disappear when the fish is placed against a white background. The green stripes may be more accurately described as being a metallic blue-green when viewed from an angle which strongly reflects light. From other angles these stripes may

appear much darker, giving a mottled blue and black appearance. The red stripes are of an orange-red hue. The markings in the male are more brilliant than in the female, especially at the breeding period. The albino (Fig. 2) is light colored and has pink eyes but shows faint orange and blue-green stripes. As will be shown later in detail, and as has been mentioned by Kosswig (1935), the black pigment is entirely absent.

Histology of the Color Pattern

The cells involved in the production of the color pattern are the black cells or melanophores, yellow cells or xanthophores, red cells or erythrophores and those containing reflecting crystals or iridocytes. There is some evidence for considering that the erythrophores develop from the xanthophores in this fish. The xanthophores contain yellow pigment granules soluble in 95 per cent alcohol in about two hours. The erythrophores contain orange-red granules less readily soluble in alcohol but sometimes xanthophores are observed which contain a few granules similar to those in the erythrophores. The erythrophores appear later in development and old fish show more red pigment than young fish.

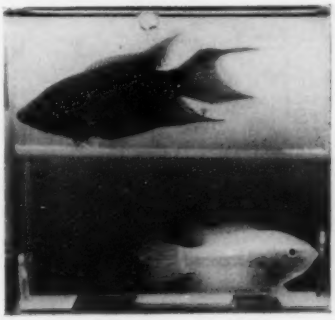

Fig. 1. Normal paradise fish.

Fig. 2. Albino paradise fish. The pictures of the two fish were taken simultaneously in one aqarium.

We recognize three zones or areas of disposition of chromatophores in relation to the individual scales. All of these are probably dermal as they lie beneath the stratified epithelial layer of the epidermis. The relationships of these areas are illustrated by Figs. 3 and 4 which show diagrammatic reconstructions of median longitudinal sections of a series of scales taken perpendicular to the body surface. These diagrams are based upon microtome sections made by three freezing methods and upon dissections of the body wall made under a dissecting binocular microscope. The first or superficial zone (*a*) (Figs. 3 and 4) lies on the upper outer surface of the scale and is connected by the second and upward sloping intermediate zone (*b*) with the third or deep zone (*c*) located on the underside of the next anterior overlying scale. This deep zone also lies above a stratum of fat cells (*d*) which separates it from the inner upper surface of the underlying scale.

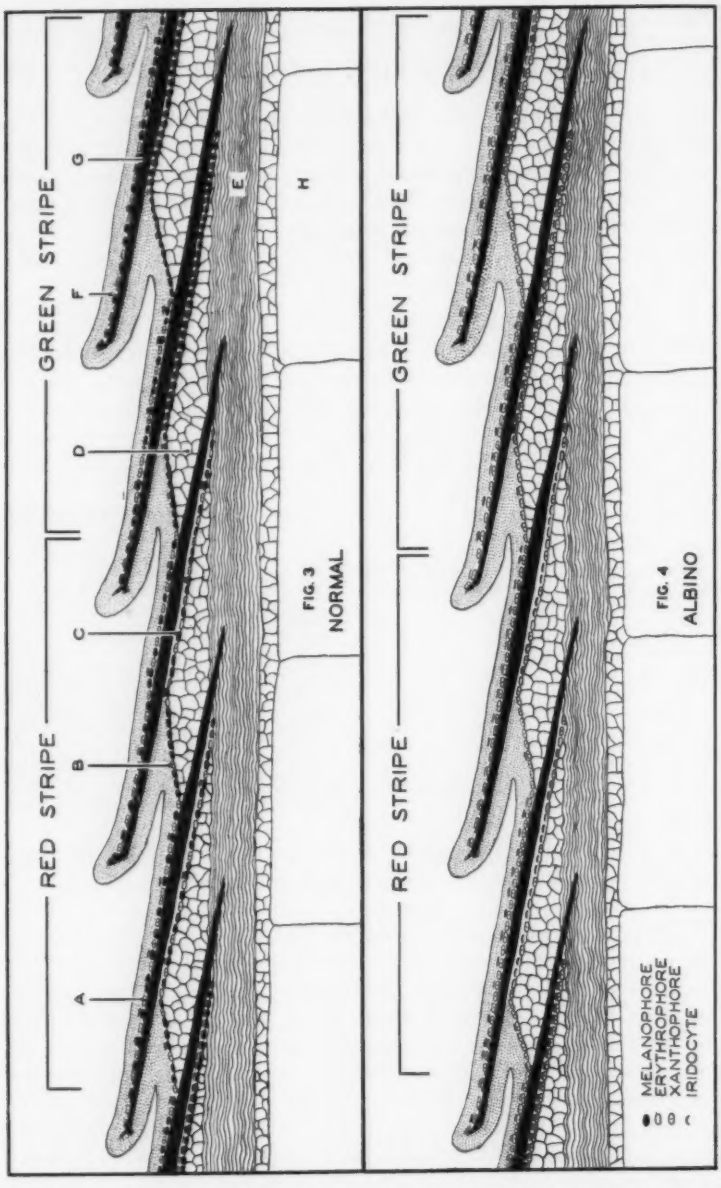

FIG. 3. Normal dark fish. Diagram of median longitudinal section of a series of scales. Magnification × 80. (*A*) Superficial zone of chromatophores; (*B*) intermediate zone of chromatophores; (*C*) deep zone of chromatophores; (*D*) stratum of fat cells; (*E*) connective tissue layer; (*F*) stratified epithelium; (*G*) scale; (*H*) muscle. Chromatophores reduced to about 75 per cent of actual number for clarification of diagram.

FIG. 4. Albino fish. Details as in Fig. 3.

TABLE II

Dark-colored fish. Counts of chromatophores in area of 0.054 sq. mm. M, melanophores, X, xanthophores, E, erythrophores, I, iridocytes.

	Superficial zone								
	Green stripe				Red stripe				
	M	X	E	I	M	X	E	I	
	22	20	0	6	15	26	0	3	
	23	18	0	3	12	20	0	2	
	17	17	0	5	13	20	0	1	
	18	24	0	4	14	18	0	2	
	19	23	0	3	18	21	0	5	
	19	17	0	3	19	20	0	0	
	25	19	0	4	20	19	0	6	
	15	22	0	2	21	26	0	3	
	18	22	0	4	21	23	0	6	
	19	18	0	0	15	25	0	7	
Average first 10....	19.5	20	0	3.4	16.8	20.6	0	3.5	Total
Average first 20....	21.2	20.2	0	3.6	18.5	20.8	0	3.6	87.9
	Deep zone								
	Green stripe				Red stripe				
	M	X	E	I	M	X	E	I	
	43		0	34	19	11	3	4	
	42		0	40	19	5	5	2	
	38		0	36	17	9	3	2	
	25		0	25	15	3	3	0	
	36		0	36	18	5	3	1	
	30		0	20	17	14	3	3	
	27		0	27	18	8	5	0	
	28		0	28	15	15	3	1	
	35		0	35	18	9	3	3	
	37		0	37	14	5	3	3	
Average first 10....	34.1		0	31.8	17	8.5	3.4	1.9	Total
Average first 20....	34.1		0	32.5	18.5	7.7	3.6	2.8	99.2

Only the first ten of twenty counts are tabulated but the average of all twenty is given.

Counts of iridocytes have a greater possibility of error than that of other chromatophores because it is difficult to determine the boundaries of individual cells.

Counts of the xanthophores from the deep zone of the dark fish (Table II) are not tabulated because they were so covered by melanophores and iridocytes that no accurate count could be made, but they are few in number or absent.

In the dark or normal type of paradise fish (Fig. 3) the upper zone contains a fairly uniform distribution of melanophores, xanthophores and iridocytes. The iridocytes tend to be superficial to and often

TABLE III

Albino fish. Counts of chromatophores as in Table II.

	Superficial zone								
	Green stripe				Red stripe				
	M	X	E	I	M	X	E	I	
	0	20	0	14	0	30	0	16	
	0	27	0	13	0	28	0	19	
	0	26	0	17	0	30	0	14	
	0	29	0	16	0	31	0	17	
	0	25	0	14	0	31	0	18	
	0	27	0	18	0	29	0	10	
	0	23	0	10	0	26	0	14	
	0	24	0	11	0	30	0	15	
	0	28	0	14	0	23	0	10	
	0	31	0	17	0	24	0	19	
Average first 10....	0	26.8	0	14.4	0	28.2	0	15.2	Total
Average first 20....	0	27.4	0	16.4	0	27.6	0	16.1	87.5
	Deep zone								
	Green stripe				Red stripe				
	M	X	E	I	M	X	E	I	
	0	17	0	20	0	13	4	18	
	0	18	0	23	0	7	3	17	
	0	12	0	19	0	8	6	10	
	0	15	0	20	0	6	5	10	
	0	20	0	17	0	10	3	21	
	0	15	0	16	0	4	5	11	
	0	21	0	22	0	13	3	22	
	0	34	0	23	0	6	4	20	
	0	31	0	26	0	11	6	15	
	0	33	0	34	0	10	4	18	
Average first 10....	0	21.6	0	22.0	0	8.8	4.3	16.2	Total
Average first 20....	0	21.7	0	23.1	0	8.2	4.4	15.4	72.8

directly above melanophores in all zones. Table II shows the relative numbers of these cells in both the green and the red stripes. The counts in the table were made on scales removed from the body and treated with adrenalin to cause a concentration of pigment in the cells

and so to help to distinguish one cell from another. The counts were of cells seen within one quadrant of an ocular counting disc. It is calculated that the area so outlined is 0.054 sq. mm. These counts will therefore when multiplied by the factor 18.4 give the approximate number of cells per square millimeter. It will be noticed that there is probably no significant difference between numbers of cells of the upper zone in the green and in the red stripes. It is the deep zone which provides the basis of the striping. Counts were made on the deep zone after removal of scales and after washing the side of the body with an adrenalin solution. It will be noted in Table II that in the deep zone the green stripes, in contrast with the red stripes, show a significant excess of melanophores and of iridocytes and but few or no xanthophores or erythrophores and that these latter are found in the red stripes. The intermediate zone very seldom contains any erythrophores and there are also fewer iridocytes than in the outer zone. It is much the same whether located in a green or a red stripe and therefore is, like the outer zone, neutral in regard to striping.

No melanophores are found in either stripe in the albino fish (Fig. 4 and Table III). Tests with adrenalin to concentrate pigment (*cf.* Goodrich, 1927) or use of the "dopa" reaction (*cf.* Goodrich, 1933) failed to indicate the presence of any "colorless" melanophores. Otherwise the disposition of cells is similar to that in the normal type except that there are more xanthophores and iridocytes.

Developmental History

The melanophores first appear at about the 16-somite stage (nineteen hours in our cultures). These are at first rather irregularly distributed and there is no indication of pattern formation until the fish is from nine to twelve days of age (about 3 mm). At this time from six to eight spots appear on both the mid-dorsal and mid-ventral aspects of the body and tail region. The striping first becomes visible in the 10 to 14 mm. fish. The number of stripes is comparable with the number of earlier spots but no certain relationship has been demonstrated.

Discussion

Histological studies on the light color phases of various fish have shown that different cellular complexes may produce these light types. In *Aplocheilus* (*Oryzias*) *latipes* a double recessive (Aida, 1921) owes its absence of color to a reduction of amount of melanin produced in melanophores and of xanthine in xanthophores (Goodrich, 1927). This circumstance in other animals has given rise to the term "color-

less" chromatophores. In this case the cells seem incapable of producing the normal amount of chromogen although sufficient oxydase is present (Goodrich, 1933). In the goldfish it is found that the light type, the transparent shubunkin, owes its condition to the early disintegration of melanophores and erythrophores (Goodrich and Hansen, 1931). In the paradise fish as described above no melanophores of any type have been seen at any stage of development. This latter form is a true albino, as no pigment is present in the eyes in contrast with the other types named above. The senior author of this paper has unpublished observations on an albino trout which has no pigment in the eyes but does have melanophores with reduced pigment as in *Oryzias*. In each case among fish so far investigated in histological detail a different developmental mechanism is concerned in producing the light phase.

Summary

1. In the paradise fish, *Macropodus opercularis*, the dark-colored or normal type is a Mendelian dominant to the albino.

2. A description is given of the cell groupings which form the basis of the color pattern.

3. The melanophores are entirely absent from the albino but all other types of chromatophores are present.

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CHROMATOPHORE REACTIONS IN THE NORMAL AND ALBINO PARADISE FISH, MACROPODUS OPERCULARIS L.

H. CLARK DALTON AND H. B. GOODRICH[1]

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The paradise fish, *Macropodus opercularis* L., was chosen for this study because there are two varieties, the normal dark type and the albino. It was thought that the albino would afford special opportunity for studying the erythrophores and xanthophores because these are not hidden by the melanophores, as they are in the dark type. These two varieties would then give a good basis for comparing the reactions of the three types of chromatophores.

The paradise fish is well known to fish fanciers. Its native habitat is the shallow coastal streams of southeastern Asia. Details in regard to the characteristics and care of this fish may be found in the book on exotic aquarium fish by Innes (1935). The albino variety apparently was first imported into this country from Germany in 1933. This variety has been shown by Kosswig (1935) to be a Mendelian recessive to the normal dark form. The histological details of the color pattern of the two varieties are described by Goodrich and Smith (1937).

Color Reactions

The adaptations of the paradise fish to various colored backgrounds have been studied by methods used by previous investigators (*cf.* Mast, 1916; Connolly, 1925, and bibliography by Parker, 1930). The fish were subjected to tests in small aquaria with white, black, red, yellow, and blue paper jackets and uniform illumination from above. Macroscopic observations on the normal dark type showed typical color adaptations to each environment. The color responses to the red and the yellow backgrounds were relatively similar but yet seemed to be definitely distinguishable. The albino fish, in which melanophores are entirely lacking (Kosswig, 1935; Goodrich and Smith, 1937), showed differing responses against black, white, red and yellow backgrounds. The greatest contrast, however, was between the very pale appearance, when placed against the white background, and the

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various slightly differing combinations of yellow, orange and red given in the reactions to the black, yellow and red backgrounds.

The results of microscopic examinations of the changes in the chromatophores are summarized in Table I. Twenty normal dark fish were available but only four albinos. Every one, however, of these albinos was tested against each background. It will be noted that in the normal dark fish the black, yellow and red cells react essentially alike to environments of black or white or red by showing the dispersed pigment in the black and red environments and concentrated pigment in the white environment. On the yellow background, however, the pigment in the black cells is concentrated, while that in the yellow and red tends to be dispersed. This gives the fish a light yellow color. The reverse situation occurs in blue surroundings, where the yellow and red cells show concentrated pigment and the black cells

TABLE I

Chromatophore behavior in the paradise fish

D = dispersed pigment; *C* = concentrated pigment; *I* = intermediate state.

	Dark variety			Albino variety		
	Mel.	Xan.	Ery.	Mel.	Xan.	Ery.
Black	*D*	*D*	*D* and *C*	—	*D*	*D*
White	*C*	*C*	*C*	—	*I*	*C* and *I*
Red	*D*	*D*	*D* and *C*	—	*D*	*D*
Yellow	*C*	*D*	*I* and *C*	—	*D*	*D*
Blue	*D*	*C*	*C*	—	*C* and *I*	*C* and *I*

dispersed pigment. The reactions of the melanophores are more rapid than those of the other two types. The erythrophores behaved in general similarly to the xanthophores, except that they were slightly slower and less extensive in their reactions.

EXPERIMENTS WITH DENERVATED CHROMATOPHORES

After the study of the color changes and their relation to the activities of the chromatophores, attention was next turned to the problem of the physiological control of the chromatophores. The methods used by G. H. Parker (1934*b*) were adopted.[2] The nerves supplying a part of the caudal fin are severed by making a cut across one of the fin rays. This operation leaves a small area between the cut and the edge of the fin without central nervous control. The

[2] Kamada (1937), whose paper has just come to hand, has also induced caudal bands in *Macropodus opercularis*.

normal dark fish lay quietly in the dish when the incision was made, but the albino required an anaesthetic, for which purpose ether was used. The experiments, except as otherwise noted, were carried out at room temperature, which remained relatively uniform, the water temperature averaging 23.5° C.

Immediately upon sectioning the nerve of a dark-colored, or normal fish the melanophores in the area between the cut and the outer edge of the caudal fin began to disperse their pigment. A clearly-defined dark band formed within one minute and it extended laterally to include half of the area between the cut ray and the two adjoining rays. When the pigment of the melanophores is dispersed, it is more difficult to observe other types of pigment cells. Nevertheless, it could be seen that the pigment in the xanthophores was also dispersed, but the reaction was somewhat slower than that of the melanophores. Three to five minutes was required for a degree of dispersion comparable to that shown in one minute by melanophores. The erythrophores lagged slightly behind the xanthophores in dispersing their pigment. These observations were confirmed later on albinos, where no melanophores obscure other types of cells.

When fish with these dark caudal bands were placed in white porcelain dishes illuminated from above, the dark bands faded in about ten hours, as the denervated melanophores gradually assumed the punctate condition. The reverse reaction, of denervated melanophores assuming the dispersed phase after concentration, is much more rapid, taking place in two to three hours.

As noted above, the xanthophores and erythrophores can be easily observed in the albino. The sectioning of a nerve was followed in one or two minutes by a detectable dispersion of pigment in these cells, which, however, take several minutes to complete the reaction. This band, although it contains no melanophores, is nevertheless striking because the rest of the fin with punctate xanthophores and erythrophores is very light. Figure 1 shows the edge of such a caudal band in the albino fish. Although these albino fish remained under the same conditions as the normal ones, there was a marked difference in that the caudal bands faded in about four hours, or less than half the time required by those in the dark-colored fish.

Certain other experiments devised by G. H. Parker (1934*c*), which have special bearing on the neurohumoral hypothesis, were tried with especial reference to the reactions of the erythrophores. The material was limited, but the results seem to be clear. Dark fish were operated on to produce a caudal band in each and then placed in white dishes until the bands were completely faded. Other cuts were then

made producing two fresh dark bands, one on either side of the distal half of the faded band. The chromatophores in the proximal half of the faded band remained concentrated, as were those in other parts of the tail (the new dark bands excepted). In the distal half, however, the chromatophores became dispersed, corresponding to the condition of their immediate neighbors in the fresh dark bands. The dispersal occurs first and to the greatest extent at the sides of the faded band. It will be recognized that these observations parallel those made by G. H. Parker on *Fundulus* and so support the theory of neurohumors as he has applied it to explain the reactions of chromatophores in fish. The humor is thought to spread from the new dark band, where it has

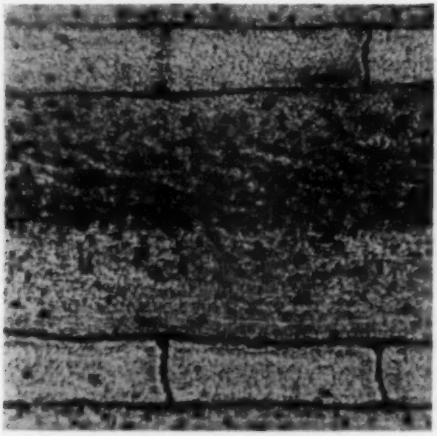

FIG. 1. Photomicrograph of edge of caudal band in albino fish. The pigment cells are erythrophores. 95 ×.

been recently liberated, to the faded band and to stimulate there the dispersion of pigment in the melanophores.

The same experiment was tried on albinos to test the occurrence of a similar dispersing neurohumor governing the erythrophores. A definite expansion was obtained in the distal half of the flanked bands in two albinos in which both lateral bands were successfully induced. It did not occur in two cases where only one flanking band was produced.

The reactions of the denervated melanophores differ from those of the xanthophores and erythrophores. This was shown by observations made when normal dark paradise fish with faded caudal bands were transferred from a white dish to a yellow environment. The melano-

phores remained concentrated, but the xanthophores and erythrophores became gradually dispersed. Each type of chromatophore assumed the same condition of pigment distribution as the corresponding type of innervated pigment cells in the surrounding area.

Temperature Experiments

As a further test of the neurohumoral hypothesis, an experiment was carried out to determine the effect of temperature on the rate of disappearance of bands produced by cutting nerves in the caudal fins of normal fish. If the fading of such bands is indeed due to the dispersion of an oil-soluble substance from cell to cell, as Dr. Parker's theory indicates, it might be expected that the dispersion should occur more rapidly at a higher temperature than at a lower one, in accordance with laws of diffusion. Accordingly, fish were operated on as previously described for the formation of dark caudal bands and isolated in white porcelain dishes illuminated from above. Two determinations were made with each fish, one at a temperature of 20° C. and the other at about ten degrees higher. The bands were considered faded when they could no longer be distinguished by the unaided eye when the fish were observed in a paraffin-lined Petri dish with a white surface in the background. The following table summarizes the data obtained:

Table II

Effect of temperature on rate of caudal band disappearance in normal fish

Observations 1–4: Low = 20° C., high = 29° C. Observation 5: Low = 23° C., high = 27° C. Figures are elapsed hours after operation when bands became so faint as to be practically indistinguishable.

	Low	High
1	18 hrs.	8 hrs.
2	10	5
3	10	5
4	10	5
5	7.75	3

The results of this experiment are what would be expected on the basis of the neurohumoral hypothesis; i.e., that the dark bands disappear more rapidly at a higher temperature than at a lower one. Furthermore, it appears that with a rise in temperature of approximately ten degrees, the time required for disappearance is reduced very nearly one half.

Discussion

The action and control of chromatophores in the paradise fish are found to be similar to that reported for certain other species. The rate of reaction of these processes, however, is strikingly different.

This disparity shows particularly in the rate of disappearance of dark bands produced by denervating chromatophores in the caudal fin. Caudal bands in *Fundulus* (Parker, 1934*a*) fade in twenty-two to ninety-six hours, while those in the catfish (Parker, 1934*b*) fade only after two or three days and may last as much as seven days. In contrast to these conditions, bands in the normal paradise fish fade in approximately ten hours, and in the albino, in about four hours. The activity of the concentrating neurohumor, then, is more rapid than in the fish previously reported.

This greater speed of action applies also to the dispersing neurohumor. Parker (1934*a*) reported that in *Fundulus* light bands, produced by transferring fish with faded caudal bands from white surroundings to black, fade in seventeen to twenty-seven hours. In the paradise fish, however, similar bands fade in only two and a half hours.

That caudal bands in the albino should disappear in approximately half the time taken by fading bands in the normal fish is a puzzling problem. Conceivably the neurohumor (or humors) produced in regulating the xanthophores and erythrophores might be more soluble than that for controlling melanophores and, consequently, become dispersed more readily than the latter. At any rate, a difference in chemical or physical properties of the two substances seems to be indicated.

The independence of action of melanophores and xanthophores is similar to that described by Abramowitz (1936) for *Fundulus majalis*. In the paradise fish, however, the erythrophores have also been studied and their reactions are found to be similar to but a trifle slower than those of the xanthophores. This gives additional evidence of the possible close relationship between xanthophores and erythrophores as suggested by Goodrich and Smith (1937).

The results obtained which show that an increase of temperature brings about an increase of rate of disappearance of the dark caudal bands may have bearing on the theory of neurohumoralism. It supports the concept that the effect is produced by a diffusing chemical substance.

Summary

1. The normal paradise fish adapts itself by appropriate color changes to environments of black, white, red, yellow, and blue.

2. Analogous but less adaptive changes occur in the albino.

3. The reactions of melanophores, xanthophores and erythrophores which produce these color changes are described.

4. Dark caudal bands, formed by cutting chromatophore nerves in the caudal fin of normal paradise fish fade in approximately ten hours. Similar bands in the albino fish fade in about four hours.

5. The evidence indicates the presence of independent dispersing neurohumors for melanophores and for erythrophores.

6. The rate of caudal band disappearance is directly proportional to the temperature, and a rise of about ten degrees in temperature very nearly doubles this rate.

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AUTOSOMAL LETHALS IN WILD POPULATIONS OF DROSOPHILA PSEUDOÖBSCURA

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INTRODUCTION

It has been recognized for some years, following the work of Muller, that lethals are especially convenient material for the study of mutation rates. This is because they occur with a frequency that is great enough to be measured, and because their occurrence can be detected by a technique that is independent of the personal equation of the observer. These same two advantages apply to the use of lethals in the study of the constitution of wild populations. Two other advantages are also to be noted in this field. Owing to the extensive studies on the mutation rates of lethals, there is available a large body of data on the frequency of the occurrence of new lethals under a variety of conditions. On the other hand, the rate of elimination of lethals from a population furnishes the minimum possible difficulty of quantitative estimation. It is, therefore, not surprising that there is a rapidly increasing literature in the field—to which the present paper belongs.

MATERIAL AND METHODS

A large series of wild strains of *Drosophila pseudoöbscura* have been studied in this laboratory (see, for example, Dobzhansky, 1935; Dobzhansky and Boche, 1933; Sturtevant and Dobzhansky, 1936). Tests have been carried out on a series of these, to determine the frequency with which autosomal lethals occur. Most of the work has been with the third chromosome, though a few tests on the second will be described below.

Wild males, or a single son from each of a series of wild females (i.e., females already fertilized when trapped), are mated individually to females carrying the recessive gene for orange eyes. From each such mating a single son is mated to females of the multiple stock orange Scute (dominant, bristle reduction) purple (recessive, eye color). All the Sc, not-or, offspring of this mating carry one particular

third chromosome from the wild specimen; when they are mated together $\left(\frac{+}{\text{or Sc pr}} \times \frac{+}{\text{or Sc pr}}\right)$ the not-Sc offspring are homozygous for one of the two third chromosomes of the original wild male. If this chromosome carries a lethal, the not-Sc class is absent.

The difficulty with the method is that, as just outlined, it neglects the possibility of crossing over. This possibility exists only in the females used in the final test generation, since everywhere else the tested chromosome is kept in males, in which crossing over is absent (or at most negligible in frequency). Even in the females in question, it happens that in the majority of cases crossing over is not a serious source of error. As reported by Sturtevant and Dobzhansky (1936), there is a wide variety of sequences in this chromosome. The or Sc pr stock has the Standard sequence; and females heterozygous for Standard and any other sequence give few crossovers. The presence of the three mutant genes in the experiment makes it possible to distinguish between Standard, on the one hand, and all the remaining sequences, on the other hand, in the tested third chromosome. In case the tested chromosome does not have the Standard sequence (and the majority of them do not have it) the presence of a lethal is at once apparent from the absence or extreme rarity of wild-type flies in the test generation. Most of the sequences do give occasional crossovers in one region or another when tested against Standard; for this reason special tests are required to distinguish between semi-lethals and lethals that cross over with Sc. These have not, in general, been carried out in the present experiments; but it is clear that very few semi-lethals are present, since in most cases the wild-type class is either wholly absent or approximately half as numerous as the heterozygous Scute class. This results from the fact that few of the lethals happened to lie in the sections that give crossovers with Scute.

If the test chromosome has the Standard sequence, lethals can still be detected, though it is necessary to make more extensive counts to be certain no lethal is present. This is due to the fact that Sc lies near the middle of the chromosome (about 30 units from the left end, 40 from the right), so that any lethal shows appreciable linkage with it. Here, however, the distinction between lethals remote from Sc, and low-viability genes close to it, requires special tests. The test, applied in most cases, has been to use a chromosome carrying the dominant Emarginate (eye shape) and the Arrowhead sequence. From the mating $\frac{\text{Em}}{+} \times \frac{\text{Em}}{+}$, results comparable to the Sc test outlined above are obtained.

In the case of the second chromosome, the test procedure is similar. Wild males or single sons of wild females are mated to glass (recessive, eye structure) females, and a single male from each such mating is mated to Bare (dominant, bristle form) glass females. The resulting Bare offspring $\left(\frac{\text{Ba gl}}{+}\right)$ are mated together, and counts are made of their offspring. This test is less efficient than that used for the third chromosome, since there is more crossing over. No inversions are present in the material tested, and Bare is about 60 units from the left end of the chromosome, 40 from the right. Lethals even at this distance do cause a disturbance of the ratios; and all suspected lethals were verified by making a series of matings of Ba × Ba from sibs. In every case these tests agreed in showing a lethal to be present.

TABLE I

Frequency of lethals

	Chromosomes tested	Lethals	
		Number	Percentage
Chromosome II			
1936	21	4	19.0
Old stocks	16	3	18.8
Chromosome III			
1935	67	13	19.4
1936	120	23	19.2
Old stocks	29	7	24.2

However, there is no test for distinguishing lethals and semi-lethals; and the technique is more laborious than that used for the third chromosome. For these reasons relatively few tests were made on the second chromosome.

The mutant strains available did not permit reasonably efficient tests for lethals in chromosomes IV or V, and none was attempted. In the case of the X chromosome no detailed tests were made, but observations on the sex ratio from wild females and their daughters indicated that no lethals were present in any of the wild strains studied.

It should be pointed out that what is being studied here is lethal-bearing chromosomes, rather than lethals. That is to say, if a chromosome carries more than one lethal the technique used does not make the fact evident. With frequencies of lethals as high as those

found it seems likely that such duplications of lethals in a single chromosome do in fact occur. The net result will be that the frequencies recorded are too low. Further complications arise in connection with the tests for identity of lethals, but since few cases of identity were found this does not seem a serious source of error.

The results obtained are shown in summary form in Table I.

The material studied in 1935 came largely from the Rocky Mountains and from the Mexican plateau; that in 1936 largely from southern California. The "old stocks" were, many of them, from the Pacific Northwest. These latter had been kept in the laboratory for at least a year before the lethal tests were begun. The striking thing about the table is that, in spite of these differences, there was little variation in the frequencies found for a given chromosome or even as between the second and third chromosomes. Table II, giving the data for the

TABLE II

	Chromosomes tested	Lethals	
		Number	Percentage
Chromosome III			
San Gabriel Canyon, Azusa, Calif.	27	8	29.6
Julian, Calif.	21	3	14.3
San Gorgonio Mt., Calif.	17	3	17.6
Santa Cruz Isd., Calif.	16	3	18.8

most-studied individual localities (included in Table I) shows a similar relative constancy of the frequency of lethals. All these results are from Race A; 5 Race B third chromosomes (all from the Olympic Peninsula of Washington) were tested, and one lethal was found, giving a frequency of 20 per cent.

As reported by Sturtevant and Dobzhansky (1936), the third chromosomes of this species exist in a wide variety of sequences. This phenomenon does not appear to be related to the occurrence of lethals. Lethals have been found in the Standard, Arrowhead, Pikes Peak, Santa Cruz, Chiricahua, Cuernavaca, and Klamath sequences. Their frequency seems to be essentially the same in regions such as southern California or southern Mexico, where several different sequences exist in fairly large numbers within each population, and in the area near the common corner of Utah, Colorado, Arizona, and New Mexico, where only the Arrowhead sequence is found. Fifteen third chromo-

somes tested from this pure Arrowhead area gave 3 lethals—exactly 20 per cent. The data on Chromosome II also indicate that diversity of sequences is not important, since all the strains from the regions concerned in these tests had the Standard Race A sequence in that chromosome.

Tests for Allelomorphism

If two lethals, different in origin, are kept in stocks of the form $\frac{\text{lethal}}{\text{or Sc pr}}$, it is easy to test their identity by crossing the two strains. If two distinct lethals are concerned, wild-type offspring will appear in numbers approximately equal to half the size of the heterozygous Scute class; if the two lethals are identical, no wild-type offspring will be produced except as a result of crossing over in the female used, and this will be infrequent if the lethal-bearing chromosome does not have the Standard sequence. Numerous tests of this kind have been carried out.

Fifteen of the 1935 and "old stock" lethals were tested in all possible combinations; the result showed 14 lethals to be present. One from Metaline Falls, Washington (1934) was identical with one from Florence, Texas (1935). Thirteen of the 1936 lethals were tested against each other, and were found to include 12 different ones. One of three tested from Julian was found to be identical with one of eight from San Gabriel Canyon. The Metaline 1934 lethal was tested against the 12 different 1936 lethals, and was found to be allelomorphic to one from Barton Flats, on the slopes of Mt. San Gorgonio, Calif. The San Gabriel-Julian duplicate was different from all the seven surviving members of the 1935 series with which it was tested. These tests, with a few miscellaneous others, total 225 crosses between separately found lethals, with 3 cases of allelomorphism. That is, an average of about $\frac{3}{225} = 1.3$ per cent of the lethals found may be expected to be allelomorphic to any given one selected at random. Or, since the total frequency of lethals in this chromosome in wild stocks is just under 20 per cent, the average frequency of a given lethal may be estimated at about one-fourth of 1 per cent.

It may be observed that no cases of recurrence have been found within a locality. That is to say, the same lethal has not been recovered from any two specimens collected in the same place. As shown above, one lethal was found both at Julian and at San Gabriel Canyon, in southern California, in 1936. While the two localities are only about

100 miles apart, the populations of their respective mountain ranges (San Jacinto and Sierra Madre) appear to be distinct in that the former contains the Santa Cruz sequence while in the latter Tree Line occurs (along with Standard, Arrowhead, and Chiricahua, common to both regions—see Dobzhansky and Sturtevant MS in press—*Genetics*). The other recurrent lethal was found in stocks from Metaline Falls, Washington (1934), Florence, Texas (1935), and Mt. San Gorgonio, Calif. (1936). It seems clear in the latter case, and probable in the former, that we are dealing with recurrent mutations rather than with the persistence of a lethal gene in heterozygous form.

Rate of Origin of New Lethals

A preliminary lethal-accumulation experiment has been carried out. The technique used is not essentially new, and the results are not yet extensive. It seems sufficient at present, therefore, to record that 2 new lethals occurred in a total of 120 chromosome-generations, giving a frequency of 1.7 per 100—with a large probable error.

Properties of Non-lethal Chromosomes

The frequency of lethals found in the tested wild stocks is unexpectedly high. A possible explanation of this result seemed to be that the species is in a condition approximating that of balanced lethals. That is to say, that most or all its third chromosomes are so constituted that flies homozygous for any one of them cannot compete with heterozygotes. Such an assumption amounts to supposing that the phenomenon of heterosis is well developed within a single pair of chromosomes. Under these conditions it might result that the rate of elimination of a lethal would be greatly decreased; since lethals are eliminated only when homozygous, and the assumption is that homozygotes play little part in the perpetuation of the species, so that their properties are unimportant.

The simplest way to test this hypothesis is to study the properties of individuals homozygous for a series of non-lethal chromosomes, from the same wild populations as those that contained lethals, in comparison with individuals heterozygous for two such chromosomes. There is another question that can conveniently be investigated at the same time—are the lethals completely recessive? This latter question is of importance in any calculation of the probable rate of elimination of lethals from wild populations.

Only one characteristic of the flies concerned has been investigated —namely their viability. It is clear that fertility, length of life, re-

sistance to unfavorable conditions, and other properties, would all be of importance in determining the frequencies found in natural populations; but the labor involved in studying a series of combinations for such a variety of characteristics has seemed prohibitive. It also seems probable, *a priori*, that there will be, in general, a rough correlation between all these properties, such that data on one of them will give a satisfactory picture of the general situation.

The technique used was the same as that used in the detection and testing for identity of lethals—in fact many of the cultures served both purposes. For the study of lethals partial counts were adequate, and were used in many cases. However, if complete counts are made on a culture there results a measure of the viability of the wild-type chromosome concerned. From the mating together of $\frac{+}{\text{or Sc pr}}$, in the absence of crossing over, three types are expected in the ratio 1 : 2 : 1. The homozygous or Sc pr is usually present in rather small numbers, and has been neglected in making comparisons, since the numbers seem to be very much influenced by minor environmental differences and are often so low as to be inconveniently sensitive to the error of random sampling. The index of viability actually used is the number of wild-type ÷ the number of heterozygous Scute × 100—i.e., the percentage that the wild type is of the Scute. Evidently, with equal viability of all classes the index will be 50.

Several objections may be raised to this method. The most serious is that each of the classes with which we are concerned is compared, not with a common standard type, but with a $\frac{+}{\text{or Sc pr}}$ class that varies in constitution from one test to another. In the case of a test of a homozygote the comparison concerns a heterozygote of that same chromosome; in the test of a heterozygote the $\frac{+}{\text{or Sc pr}}$ class is made up of heterozygotes for each of the two + chromosomes, in approximately equal numbers. In a few cases tests were repeated, and the result shows a definite, though far from complete, correlation between successive tests of a given combination. The use of this technique can, therefore, not be considered as giving more than an indication of the situation, and accordingly it does not seem desirable to present the results obtained in detail.

In general, the homozygotes for non-lethal third chromosomes from wild stocks are viable and fertile in both sexes, with the result that homozygous stocks are easily established. The test outlined above

indicates that some homozygotes are definitely below par, others apparently at no disadvantage. The average viability index of 21 tested chromosomes was 45.91, the minimum 19.4 (weighted average of three tests—16.3, 21.6, 33.3).

The tests of heterozygotes for two-third chromosomes from wild strains were made chiefly with lethal-bearing chromosomes. Twenty-five different combinations of lethal × lethal gave an average index of 53.0—i.e., more viable than the separate lethals heterozygous for or Sc pr. The lowest index among the 25 was 38.3.

The most probable conclusions are that

(1) lethals are wholly recessive;
(2) homozygotes for non-lethal chromosomes are slightly less viable, on the average, than heterozygotes;
(3) the viability of homozygotes is quite variable, some being definitely low, others apparently as high as the heterozygotes.

Comparison with Results from D. melanogaster

The frequency of lethals in wild populations of *D. melanogaster* has been studied by several authors. The most extensive work is that of Dubinin and co-workers (1934, 1936) on material from the Caucasus. These studies were carried out on a large scale. Altogether 4819 second chromosomes are recorded, with 470 (= 9.8 per cent) lethals. The frequencies range from 0 (in 92 tested, from Delizhan) to 16.1 per cent (161 tested, from Ordzhonikidze). From one locality, Gelendzhik, tests were made in three successive years, giving 7.98 per cent (877 tested), 12.86 per cent (616 tested), and 8.78 per cent (797 tested). Tests were also carried out for identity, but only within regions. The average value here was 2.2 per cent—i.e., an average of 2.2 per cent of the lethals found in one year at one locality may be expected to be allelomorphic to any one lethal found in that year at that locality. The average frequency for a given single lethal is 0.22 per cent (.022 × .098). There are no tests for identity of lethals from different localities, but those found at Gelendzhik in successive years were so tested, with the result that "among the 33 lethals of 1933 and the 55 lethals of 1934, nine lethals were common to both years." The conclusion is drawn that this represents survival of these lethals. The results reported in the present paper throw some doubt on this conclusion, for a comparable frequency of identity was found in *pseudoöbscura* for all years and all localities. One may surmise that, in *melanogaster* also, any two series of lethals would show a fairly large proportion of common members—i.e., that recurrent mutations are relatively frequent.

The absolute frequency of lethals found in the Caucasian *melanogaster* populations is only about half that in the American *pseudoobscura*, though the chromosome concerned (II) is roughly twice as long as the III of *pseudoöbscura* and includes the same material plus that of the *pseudoöbscura* IV (Donald, 1936; Sturtevant and Tan, 1937). The preliminary experiment described above suggests a higher lethal mutation rate in *pseudoöbscura;* but until more data are available on this point it is scarcely possible to decide on the relative rates of elimination of lethals in the two species.

One difficulty in evaluating the data of Dubinin and his co-workers may be pointed out. The results are recorded by localities and years; but we are nowhere given more specific data. How large an area is included in a single "locality"? Over how long a period were specimens taken in one year? What kinds of places were collected—woods, grocery stores, garbage dumps, fruit orchards? These questions are of importance in judging the size of the populations sampled and the probable degree of relationship of the tested individuals.

Summary

1. Approximately 20 per cent of the third chromosomes found in wild populations carry lethals.

2. Less extensive data indicate a similar frequency for the second chromosome.

3. On the average, about 1.3 per cent of the lethals found may be expected to be allelomorphic to any given one.

4. The average frequency for any one lethal is about one-fourth of one per cent of the chromosomes of wild strains.

5. The lethals, so far as studied, are completely recessive.

6. Study of flies homozygous for non-lethal third chromosomes shows a considerable variation in their viability. Some are at a definite disadvantage, others apparently not. On the average they are not quite as viable as flies carrying two different third chromosomes.

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THE EFFECT OF SALINITY UPON THE GROWTH OF EGGS OF FUCUS FURCATUS[1]

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INTRODUCTION

The effect of salinity upon fertilization and early development in 3 species of *Fucus* (*F. serratus*, *F. vesiculosus*, and *F. spiralis*) was studied by Kniep (1907) on the Norwegian coast. He investigated especially the tolerance for dilution of the sea water and found it to differ considerably in the different species. Kniep was able to correlate the tolerance with the distribution of the species (and, by inference, of additional species of *Fucus* and other related marine algæ as well) especially in the Baltic Sea and in near-by regions in which brackish water communicates with the ocean, thus establishing gradients of salinity.

The present investigation is an attempt to measure the effect of increased and decreased salinity upon the percentage germination and the growth rate of the fertilized eggs of *Fucus furcatus f. luxurians* throughout the range of salinity in which germination and growth take place, and to do so under strictly controlled conditions. The eggs were transferred abruptly into sea water of altered salinity, and therefore, any effect which a prolonged gradual adaptation might have on the tolerance is eliminated from the measurements. The data are useful in conjunction with other experimental investigations of the development of this egg in which the salinity of the medium varies.

MATERIAL AND METHOD

Fruiting tips of this hermaphroditic *Fucus* were collected at Moss Beach, California in May, June and July, 1937. The material was cared for in the manner described in an earlier paper (Whitaker, 1936). Sea water was collected at the same place and was filtered twice upon arriving at the laboratory. In each experiment, eggs from the same batch were reared in this sea water and also in samples of the same sea water with artificially altered salinity. Salinity was decreased by adding triple glass-distilled water. It was increased by boiling under

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vacuum so that the temperature did not rise enough to cause precipitation of salt. By this method the volume of sea water was reduced to half without precipitation. The measure of salinity which has been used is the total salt content per unit volume expressed as a percentage of the total salt content per unit volume of normal sea water. In all cases hydrometer readings were made which confirmed the salinity as originally determined volumetrically. The specific gravity of the normal sea water used was 1.027.

The pH of the samples of normal sea water, as determined with a glass electrode, ranged from 7.9 to 8.2. The pH tended to rise slightly when the salinity was artificially increased, and to fall when it was decreased. Such shifts were usually not great, being of the order of a few tenths of a pH unit or less in most experiments. In the most extreme cases the shift amounted to 0.5 units. The maximum pH range of the media in the experiments upon which Fig. 1 is based was 7.7 to 8.5. In some of the experiments, all of which gave essentially similar results, the range was considerably less. This pH range is much narrower than the limits of normal development for this species of *Fucus* (Whitaker, 1937), and it is small enough so that it appears safe to conclude that it is a minor factor in the present results.

Since *Fucus furcatus* is hermaphroditic, and sheds male and female capsules together at the same time, it is not feasible to inseminate a population of eggs at any precise moment. The male capsules dissolve in the sea water first, however, so that fertilization takes place when the egg capsules dissolve and liberate the individual eggs into the sperm bearing sea water. By selecting eggs only from capsules which break down during a limited period, it is possible, in effect, to confine fertilization to this period. In these experiments eggs were used which were fertilized during a 10-minute period.

In order, for the present purpose, to rule out the effects of altered salinity upon fertilization and entrance of the sperm, eggs were fertilized in normal sea water in all experiments. Twenty minutes after the end of the fertilization period samples were transferred with a negligible amount of normal sea water into the media of altered salinity. Eggs had thus been fertilized 20–30 minutes when they were removed from normal sea water. They were thinly seeded in covered Petri dishes which were stored in a moist chamber in a dark, humid, constant temperature room at 15° C. Fertilization and all subsequent development took place in this constant temperature room. The eggs were observed only with red light until the end of the experiment.

Results

The fertilized *Fucus* eggs develop in a relatively great range of salinity. As the limits of tolerance are approached, all eggs in a population do not have the same end point, and the results are represented graphically by the blocks in Fig. 1 to show the percent of the eggs in the populations which form rhizoids. Each block in the figure represents the average of the results of from 4 to 10 experiments, each involving counts on 300 or 400 eggs. The counts were made two days after fertilization.

The blocks in Fig. 1 show that very high percentages of the eggs

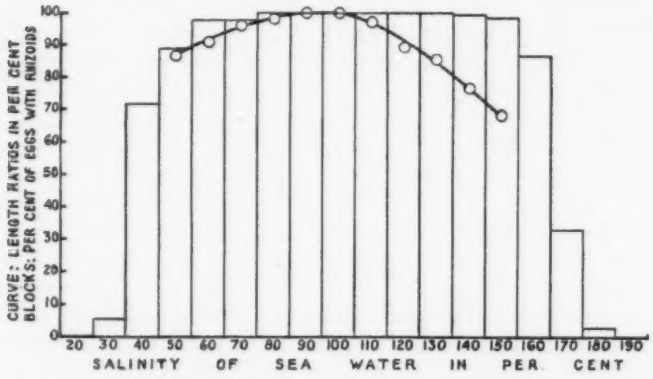

Fig. 1. Blocks: The percentages of eggs which formed rhizoids in normal sea water, in diluted sea water and in concentrated sea water, as observed after two days.

Curve: The average over all length of embryos at 92–96 hours, reared in sea water of the salinity indicated, expressed as a percentage of the average length of control embryos reared in normal sea water.

In all cases the eggs were fertilized and reared in the dark at 15° C. The specific gravity of the normal (100 per cent) sea water was 1.027 (see text).

develop in sea water ranging in salinity from 50 or 60 to 150 per cent of that of normal sea water. Beyond these limits the percentage drops off rapidly, although some eggs develop rhizoids when the salinity is as extreme as 30 per cent or 180 per cent. Beyond these limits no rhizoids are formed. In the more dilute sea water (10 per cent and 20 per cent) eggs burst and cytolyze at once, and many of the eggs which fail to form rhizoids in 30 per cent or 40 per cent sea water also cytolyze. In the concentrated sea water, on the other hand, most of the eggs which fail to form rhizoids do not cytolyze, at least in the course of several days. Eggs which have remained for two days in sea water of twice the normal salinity form rhizoids after being returned

to normal sea water. The suppression at high salinity thus bears some resemblance to anesthesia.

The *Fucus* egg is spherical until a bulge forms on one side, about 16 hours after fertilization (Whitaker, 1936). This bulge extends in filamentous fashion by elongation and cell divisions until its length is many times the diameter of an egg. Ultimately the rhizoid of the new plant forms from this filamentous structure, while the remainder of the embryo gives rise to the thallus. The rate at which early development takes place, as indicated by the rate of extension of the rhizoid filament, is a function of the salinity of the medium. In both extremes of the range of salinity, in which many or most of the eggs form no rhizoids at all, the rate of extension is greatly retarded in those eggs which do form rhizoids. Within the ranges of salinity (50 or 60 to 150 per cent, see blocks, Fig. 1) in which practically all of the eggs form essentially normal rhizoids, the salinity affects the rate of extension as shown by the curve in Fig. 1.

The curve in Fig. 1 shows the average full length of the embryos reared in media of the salinities indicated, expressed as a percentage of the average full length of the control embryos from the same batches of eggs reared in normal sea water. The lengths were measured with an occular micrometer 92–96 hours after fertilization. Each point was obtained by averaging the results of 4 to 10 experiments, each involving counts of 50 or 100 eggs, except that the point at 90 per cent salinity is based on only 3 experiments. The results of the separate experiments are very similar.

It is seen from the curve in Fig. 1 that the embryos grow as rapidly in 90 per cent as in 100 per cent sea water. The average absolute length of the embryos in these optimum salinities was 303 microns at 92–96 hours. Since the embryos were growing in the dark in the absence of photosynthesis, no nutrients were available to the eggs except those stored in the unfertilized eggs. It is therefore doubtful if much new protoplasm was synthesized, although internal conversion of stored foodstuffs may have supported some protoplasmic synthesis. The growth or extension observed is presumably largely a developmental elaboration, i.e., an extreme change of form.

Summary

1. Fertilized eggs of *Fucus furcatus f. luxurians* have been reared in the dark at 15° C. in diluted and concentrated sea water.

2. When the salinity of the medium is between 60 per cent and 150 per cent that of normal sea water (sp. gr. 1.027), practically all of the eggs in a population form rhizoids and develop. The rate of elongation

of the embryos, as measured at 4 days, is the same when the salinity is 90 per cent or 100 per cent. When the salinity is greater or less, the growth rate is retarded as shown in the curve in Fig. 1.

3. As the salinity is reduced below 60 per cent or is increased above 150 per cent, the percentage of eggs which form rhizoids declines rapidly as shown in the blocks in Fig. 1. In 10 per cent and 20 per cent sea water the eggs burst and cytolyze. In concentrated sea water which inhibits development the eggs do not cytolyze and the developmental inhibition may be reversible.

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STIMULATION AND NUCLEAR BREAKDOWN IN THE NEREIS EGG[1]

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When a protoplasmic system is exposed to a so-called stimulating agent, a series of changes occurs which results eventually in activity of the protoplasm. Various opinions have been expressed as to the physico-chemical basis of stimulation. In our laboratory for several years we have been interested in proving that stimulating agents all cause a release of calcium from a calcium proteinate gel in the cell cortex. This calcium is then supposed to initiate a protoplasmic clotting similar in some ways to blood clotting. The evidence on which this theory is based has been summarized in a recent book (Heilbrunn, 1937, Chapter 37), and the details of the theory are also given there.

Although in previous studies various types of animal and plant material have been used, and the theory has thus been applied to cells very different morphologically, as yet no attempt has been made to determine what effect stimulation may have on the cell nucleus. Certainly in a dividing cell, the nucleus plays an all-important rôle. It is of interest, therefore, to inquire what effect stimulation may have in those cases in which the primary result of stimulation is cell division. As is well known, the egg cells of many marine invertebrates are sensitive to a wide variety of stimulating agents, and following stimulation, division is usually initiated.

Typically, during mitosis the nuclear membrane breaks down. The mechanism of this breakdown has scarcely been investigated experimentally, although an understanding of the causes of such nuclear breakdown would be an important link in any complete theory of cell division. In the case of many marine invertebrates, the egg is released into the sea water in an immature condition, and it contains a large nucleus or germinal vesicle. Sometimes contact with sea water is in itself sufficient to cause a breakdown of the germinal vesicle and an initiation of the maturation or polar body divisions. This is true of the starfish egg. In other instances, the egg remains immature and retains its large germinal vesicle until insemination. Then the

[1] This work has been aided by a grant from the Radiation Committee of the National Research Council.

entrance of sperm into the egg, or its contact with the egg cortex, provides the stimulus for nuclear breakdown and maturation divisions. The egg of the annelid worm *Nereis limbata* has a large germinal vesicle when it is shed into sea water, and this is broken down after insemination. The *Nereis* egg has been widely used both in studies of normal fertilization (Lillie, 1911), as well as in studies of artificial parthenogenesis (Just, 1915; Heilbrunn, 1925). It is readily obtainable at Woods Hole, and is an easy object to study. Following activation, the breakdown of the germinal vesicle can readily be detected in the living egg, especially if the egg is somewhat compressed between slide and coverglass.

Some years ago, Just showed that the *Nereis egg* could be artificially activated by heat (Just, 1915; see also Heilbrunn, 1925). Later, Just (1933) found that ultra-violet radiation could also cause activation. In general, it is believed by radiologists that Roentgen rays can produce the same effect as ultra-violet radiation. Accordingly an attempt was made to discover if activation could be produced by Roentgen rays. It was found that in order to obtain results, extremely high dosage was necessary. In three experiments in which eggs were exposed to 7600 r units per minute for 75 minutes, the percentage of eggs activated was found to be 74, 52, and 5. Three other experiments gave negative results. The activation was not the result of heat, for the dishes containing the eggs were packed in ice to keep them from becoming overheated.

The *Nereis* egg may be activated by chemical agents as well as by physical means. It was found that isotonic solutions of sodium chloride cause a high percentage of germinal vesicle breakdown. Similar effects may also be obtained with isotonic solutions of potassium chloride. Doubtless various other chemical agents are also effective, but no attempt was made to list all the effective reagents. However, it may be noted that a dilute ether solution (3 per cent in sea water) is somewhat effective in causing germinal vesicle breakdown. Other fat solvents doubtless act in similar fashion.

The literature on artificial parthenogenesis contains many papers in which activation of marine eggs has been found to follow various types of treatment. Our concern has not been to prepare an exhaustive list of the effective agents for the *Nereis* egg. We have attempted to discover the nature of the effect of several physical and chemical activating agents, and we have assumed that if we can throw light on the true mechanism of activation in two or three cases, such information may perhaps be applied to all types of activation.

In our experiments we have studied the action of ultra-violet

radiation and of isotonic salt solutions, and we have tried to discover whether the calcium release theory of stimulation will hold for these cases. Our experimental procedure was very simple. First we determined the percentage of germinal vesicle breakdown following exposure to the stimulating agent. Then we immersed eggs in citrate solutions, to discover if previous exposure to citrate would prevent activation. As is well known to students of blood clotting, citrate solutions tend to prevent the action of calcium. Opinions as to the mechanism of the effect differ. (Compare Hastings, McLean, Eichelberger, Hall and DaCosta, 1934.) Some unpublished work of D. Mazia indicates that when sea-urchin eggs are immersed in citrate

TABLE I

Nuclear Breakdown following Irradiation

Percentage breakdown after 60 seconds irradiation in sea water	Immersion time in 0.35 M Na citrate	Percentage breakdown after 60 seconds irradiation in Na citrate
	minutes	
100	3½	0
100	4	7
98	4	7
100	4	2
100	4	6
40	4	5
100	4	2
100	4	37
92	4	11
97	4	5
100	5	0
100	5	5
100	5	0
97	5	19
97	29	0

solutions, a large percentage of the calcium normally present in the cell cortex is removed. If citrate solutions are able to remove calcium from the cell cortex, or if they prevent release of calcium to the cell interior by some other mechanism, then citrated eggs should be incapable of activation. This was found to be the case.

Two types of experiments were tried. In the first place, eggs were exposed to ultra-violet radiation, both in sea water and in sodium citrate solutions. Ultra-violet radiation was obtained from a Uviarc Laboratory Outfit. This type of lamp is well known and its characteristics have been described in a paper by Buttolph (1930). The lamp was operated at 160 volts and 5 amperes. In our work we were not interested in the energy output of the lamp nor in separating

different wave-lengths of radiation. A few preliminary tests soon showed that when *Nereis* eggs were placed in a small quantity of sea water and exposed to the mercury arc at a distance of 26 cm. for 60 seconds, all of the eggs, or at least more than 90 per cent of them, showed activation. Shorter exposures (e.g. 30 seconds) were not quite so successful. On the other hand, following exposures of 3 or 4 minutes, slightly lower percentages of activation were obtained. It is thus clear that under the conditions of our experimentation, a 60-second exposure to the Uviarc lamp was an effective stimulus, and we have reason to believe that other Uviarc lamps act approximately as ours did, unless of course, the lamps have suffered very serious deteriora-

TABLE II

Nuclear Breakdown in Isotonic NaCl

Percentage breakdown in NaCl	Immersion time in 0.35 M Na citrate	Percentage breakdown in NaCl following citrate treatment
	minutes	
99	4	4
96	4	4
99	4	7
93	4	24
35	5	0
86	6	2
88	6	0
69	6	0
89	6	0
100	6	0
100	6	1
100	6	0
95	6	0
94	6	1
99	6	0
100	8	2

tion. In any case, it is a simple matter to determine the exposure necessary to obtain activation, for there is a wide range over which this occurs.

Table I shows the percentages of nuclear breakdown following irradiation in sea water, as compared with the percentages obtained following irradiation in citrate solutions. The difference is striking. In sea water typically, practically all the eggs respond by nuclear breakdown. On the other hand in the citrate solution, there is almost no response.

Similarly, when eggs are exposed to isotonic sodium or potassium chloride solutions (0.53 molar), there is a high percentage of response, but if the eggs are first immersed in 0.35 molar sodium citrate and then

exposed to the sodium or potassium chloride solutions, the eggs fail to respond. This is shown in Tables II and III. From these tables it will be noted that although a 4-minute immersion in the citrate solution is sufficient to prevent most of the eggs from showing nuclear breakdown, occasionally there is some response following such immersion. Thus in the fourth experiment of Table II, 24 per cent of the eggs were activated after 4 minutes of citrate treatment. If the eggs are allowed to remain in the citrate solution for 6 minutes before being transferred to the sodium chloride or the potassium chloride solution, there is almost no nuclear breakdown. Apparently, the

TABLE III

Nuclear Breakdown in Isotonic KCl

Percentage breakdown in KCl	Immersion time in 0.35 M Na citrate	Percentage breakdown in KCl following citrate treatment	
	minutes		
40	4	18	
93	4	10	
100	4	Some eggs broken up.	Count impossible.
100	4	Some eggs broken up.	Count impossible.
52	5	0	
93	6	1	
28	6	0	
11	6	0	
81	6	0–2	
44	6	0	
71	6	0	
43	6	0	
99	6	0	
100	6	0	
99	6	0	
100	8	4	

citrate solution must act 5 or 6 minutes if it is to be completely effective in preventing response to stimulation.

In these studies of activation, our attention was focussed on the nuclear breakdown, and no attempt was made to study the cortical changes which occurred in the eggs. These changes are interesting, but they were not considered in the present study.

It might well be urged that the effect of the citrate in preventing the stimulating effect of ultra-violet radiation and of sodium and potassium solutions was essentially due to injury. Thus, if eggs are fixed in formalin or some other poison, they presumably would not respond to any type of stimulation. This objection is very easily met. If citrated eggs are returned to sea water, they immediately become

sensitive to radiation again. This is clearly shown in Table IV. Eggs exposed to citrate for 6 minutes and then returned to sea water show nearly 100 per cent of germinal vesicle breakdown on irradiation. In this connection, it should be pointed out that even without irradiation, a certain percentage of eggs may show germinal vesicle breakdown after transfer from the citrate solution to sea water. This is an interesting fact and will be considered again later.

As a whole, our results show that both physical and chemical stimulation of the *Nereis* egg, as evidenced by the breakdown of the germinal vesicle, are ineffective if the eggs are first treated with citrate. There is thus support for the theory that stimulation to be effective must involve a calcium release from the cell cortex. It is rather easy to understand why ultra-violet radiation might cause a breakdown of a calcium proteinate gel with consequent release of calcium, for this

TABLE IV

Nuclear Breakdown resulting from Irradiation in Sea Water following Citrate Treatment

Immersion time in 0.35 M Na citrate	Immersion time in sea water before irradiation	Percentage breakdown following irradiation
minutes	*minutes*	
6	4	92–95
6	4	98
6	4	98
6	10–10½	99
6	10–10½	100
6	10–10½	98
6	10–10½	100
6½	3½	97

is consistent with various types of earlier work both on protoplasm and on proteins (for a review of this literature, see Heilbrunn and Mazia, 1936). On the other hand, it is rather paradoxical to assume that immersion of a cell in pure sodium or potassium chloride solution would cause a release of free calcium to the cell interior. However, there is real evidence that this type of phenomenon does actually occur (see Heilbrunn, 1937, Chapter 33).

If *Nereis* eggs are immersed in isotonic calcium chloride solutions (0.29 molar) there is no breakdown of the germinal vesicle. Magnesium solutions are also without effect. This apparently means that the calcium ions are unable to penetrate the cell with any rapidity, although this is not the only possible explanation. In the case of the clam *Mactra*, some unpublished experiments of Miss R. A. Young show that activation (which in this egg also involves germinal vesicle

breakdown) follows immersion in calcium chloride solutions. (See also earlier work of Dalcq, 1925, 1928; Hörstadius, 1923; Hobson, 1928; Pasteels, 1935.) From these studies with marine eggs we can conclude that for some, calcium chloride solutions are stimulating agents, whereas for others calcium chloride solutions are without any very apparent effect. This is entirely comparable to the situation with respect to smooth muscle. Thus Tate and Clark (1922) showed that calcium caused a contraction of the uterine smooth muscle of the rabbit and cat, but had no effect on similar muscle in the guinea-pig and rat.

In the case of *Nereis*, the eggs become sensitive to calcium chloride solutions and to a lesser extent to sea water, following immersion in sodium citrate solutions. Thus if eggs were immersed in 0.35 molar sodium citrate for 60–65 minutes and then placed in 0.29 molar calcium chloride, 80 to 100 per cent of them showed germinal vesicle breakdown (four experiments gave percentages of 80, 100, 92, 82). It almost appears as though the loss of calcium from the cell cortex makes it possible for calcium to enter the cell more freely. It is possible that there may be a relation between this phenomenon and the calcium paradox as observed in invertebrate hearts (see, for example, Chao, 1934).

Our results lead us to conclude that the breakdown of the germinal vesicle in the *Nereis* egg is the result of a release of calcium from the cortex. Previously Dalcq had suggested that the calcium ion was responsible for the breakdown of the germinal vesicle in the starfish egg, but he offers no clear explanation as to why sodium or potassium should act like calcium, either in the starfish egg or in other eggs. (See Dalcq, 1925, 1928; also Pasteels, 1935.) Our results offer an interpretation of this difficulty. As to the details of the mechanism involved in the disappearance of the nuclear membrane, we are still at a loss. Presumably, calcium ion initiates a series of changes and these changes eventually cause nuclear breakdown. There is obviously more than a mere action of calcium on the nucleus, for when eggs are crushed in sea water, the germinal vesicle does not break down.

Summary

When the *Nereis* egg is stimulated either physically by ultra-violet radiation, or chemically by immersion in sodium or potassium chloride solutions, the germinal vesicle breaks down. This response to stimulation is prevented if the eggs are previously immersed in sodium citrate solutions. The results are in agreement with the calcium release theory of stimulation.

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STUDIES OF THE MITOTIC FIGURE. VI. MID-BODIES AND THEIR SIGNIFICANCE FOR THE CENTRAL BODY PROBLEM

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INTRODUCTION

Purpose of the Study

Mid-bodies have received less attention than any other component of the achromatic figure. In many animal cells they appear, during the time of division, as deeply staining thickenings on the fixed spindle fibers, in the equatorial plane. They are generally regarded as in some way comparable to the cell-plate of plants—possibly a vestigial homolog (Wilson, 1928, pp. 144 and 159; Sharp, 1934, p. 169). Flemming (1891) first suggested this concept.

In a previous study of central bodies in brain cells of *Squalus* embryos, in which considerable attention was also given to the mid-bodies, I reached the conclusion that in this case they are nothing but accumulations of dye at places where the spindle materials have been pinched together by the division furrow, and have no existence as an individualized cell component. The question was raised: "Whether or not mid-bodies generally will eventually be explained in terms of focalization phenomena remains to be seen" (1933*a*, pp. 177–178). This problem is the subject of the present paper.

Discussion of the Term "Focal Body"

Before presenting the data it is advisable to clarify further the concept *focal body* or *focalization phenomenon* which I have discussed previously (1932, pp. 181–182; 1933*a*, p. 177; and 1933*b*, pp. 233–235).[1] These terms are used to describe a structure of the achromatic figure which appears to be produced as a result of the convergence to a common center of either spindle materials, aster materials, or both. If this assumption is correct, there are three possible classes of focal bodies.

Living Focal Bodies.—These probably exist in the living cell as minute pools of spindle or aster materials at centers of focalization, and are preserved by fixation. The situation here may be somewhat like

[1] Some of these studies, referred to here and later, were carried out with the assistance of other investigators, as indicated in the bibliography.

that which occurs when thin lines of a substance of relatively low viscosity are placed upon a piece of glass in a converging configuration: a pool of material forms where the lines coalesce at the center, the size of the focal body thus produced depending upon the number and thickness of the lines, and the viscosity of the substance. Such a body is non-radial and more or less demarked, but although it looks very different from the surrounding radial area and appears to be an individualized structure, it is actually only the inner ends of the lines which have fused.

Coagulation Artifact Focal Bodies.—According to the hypothesis presented here and in earlier papers, these do not exist in the living cell but are created by the act of coagulation. It is suggested that the process of fixation may effect a breakdown of the converging materials at the focal center and form a body, the structure of which is determined largely by the coarseness of the fixed rays and fibers. As I have shown in previous studies, the comparative coarseness of such materials is modified by the technique—the fixative used or the environmental condition prior to fixation. For example, in eggs of *Echinarachnius* (1928, 1929*b*, and 1929*c*) and *Chætopterus* (1932 and 1933*b*) there is a definite relationship between the structure of bodies at centers of focalization and the coarseness of the fibers and rays, as determined by the technique. An analogy to this situation is found in a model similar to the one described above, but employing material of such a composition that the converging lines do not coalesce at the point where they meet. Upon subjecting this model to a given treatment (the equivalent of the fixation procedure), the character of the material is so changed that the lines coalesce at their meeting-point and a focal body is formed.

Staining Artifact Focal Bodies.—These are only accumulations of dye at the focal centers of some fixed mitotic figures, such as I demonstrated in brain cells of *Squalus* embryos (1933*a*). An analogous situation occurs if a model is made in which a large number of delicate threads are attached to the edge of a circular frame and brought together at the center. When such a model is dyed, the focal area takes more stain than the peripheral region, owing to the aggregation of threads there. If this model is viewed at the proper distance (duplicating the effect produced when stained mitotic figures are studied with a microscope) the central region may in some cases look like a sharply demarked body, depending upon the number of threads, the chemical nature of both threads and dye, whether they are thick or thin, their surface rough or smooth, and their paths separate or interwoven.

Assuming that the suggestion is correct that the focalization of spindle and aster materials may result in the production of focal bodies, we still know too little about the structure of living spindles and asters, the effects of coagulation, and the staining process, to explain adequately in physico-chemical terms exactly how the bodies are produced. For these reasons the models just described are not intended to give exact parallels of what occurs when focal bodies are formed in mitotic figures; they only illustrate how focalization phenomena may be involved in any configuration of converging substances.

Depending upon the type of cell used and the technique employed, such bodies may be minute or large, demarked sharply or vaguely, granular or homogeneous or vesicular in structure, and they may differ in shape. Such variations can often be demonstrated in the same material when different techniques are used.

Focal bodies, however, have certain characteristics by which they can be surely differentiated from typical cell components. They exist only at places where spindle and aster materials come to a focus. They arise only as such places of focalization are formed, and disappear when such areas disintegrate; they therefore do not maintain continuity from cell to cell. They change shape as the focal area changes shape, and often become double when the focal area elongates. They may vary widely in structure from one cell cycle to another in the same organism, or they may vary in the same cell cycle in closely related organisms; sometimes, on the same slide, they show differences from cell to cell which are identical as to species, cell cycle, mitotic phase, and the method used. They are usually unstable in structure and easily modified by variations in the technique. In general their morphology is related to the size of the mitotic figure and the coarseness of the fixed focalized materials.

In all of these respects focal bodies differ markedly from such cell components as the centriole-blepharoplasts of spermatocytes and Protozoa, the diplosomes of vertebrate cells, and other similar structures, which have undoubted existence in the living cell as typical individualized cell components, and in some cases can be seen in the living condition. They exist regardless of whether areas of focalization are present or absent, and they exhibit great stability of structure despite wide variation in the technique.

Hence the suggestion is made that at centers of focalization, structures are formed which are often minute, homogeneous, sharply demarked, and look like typical cell components, but are actually nothing more than the result of the convergence of spindle and aster materials to focal areas, i.e., *focal bodies* or *focal phenomena.*

Methods

The behavior of mid-bodies was studied in eggs of *Cerebratulus*, *Cumingia*, *Nereis*, *Chætopterus*, and *Asterias*, during the formation of both polar bodies, during first cleavage, and also in cells of blastulæ; in *Arbacia* eggs, during the first three cleavages as well as in blastulæ; in brain cells of *Squalus* embryos; and in primary spermatocytes of *Romalea*.

Many fixatives were used and the cells were exposed to various environmental modifications prior to fixation. They were sectioned at a thickness of 5 μ and stained with Heidenhain's hæmatoxylin. In the case of eggs mid-bodies were studied only in those which happened to be sectioned in a plane passing through both the long axis of the mitotic figure and the animal pole, since only in such sections are the relations between the structure of mid-body and spindle shown clearly. In each case an adequate number were examined to ascertain the extent of variation occurring in mid-body structure and associated spindle remnant.

In order to check the conclusions reached by these original studies I also examined a considerable portion of the pertinent literature—almost two hundred papers—to be sure that no important types of mid-bodies described by other workers should be overlooked. In most of these investigations the author was not interested in the mid-bodies; in many cases they are not even mentioned in the paper, although they are shown in the drawings. In only a few studies do the figures delineate the detailed changes from their origin to their disappearance.

The illustrations of the present paper, whether original or reproduced from the literature, show all types of mid-bodies found, typical as well as atypical.[2]

The Behavior of Mid-bodies

Conditions Under Which Mid-bodies Are Present

Mid-bodies arise only when a remnant of the spindle still exists when cell division occurs; this is necessarily pinched together by the advancing furrows. No mid-bodies are found if, as often happens, the spindle disappears prior to division.

Such contrasting conditions may occur in the same cell type of closely related species, as illustrated in cleaving eggs of Echinoidea. In a series of experiments, done in collaboration with Dr. G. H. A. Clowes

[2] Those illustrations taken from the literature (Figs. 13–69) were for the most part reproduced by photographing the drawings of the original papers. In four cases, however (Figs. 14, 62, 65, and 66), photographs were made of illustrations, redrawn from the originals, in Wilson's *The Cell in Development and Heredity*.

and Dr. M. E. Krahl, I studied *Arbacia* eggs under about 75 experimental conditions, in an attempt to analyze the relation between modifications of respiration and the behavior of the mitotic figure. Usually the eggs were fixed in Bouin's fluid, but a number of other reagents were used. Mid-bodies are present (Fig. 5) with a spindle remnant (with rare exceptions when the material is very abnormal and both are absent). Boveri (1901) illustrates a similar condition in cleaving eggs of *Echinus* (Fig. 59). On the other hand, *Echinarachnius* eggs were also studied, using about 20 modifications of the technique, and the situation is in contrast to that in *Arbacia* eggs: the spindle disintegrates before division occurs and mid-bodies are not found. *Toxopneustes* eggs (Wilson and Leaming, 1895) also show neither body nor remnant. Here, therefore, are eggs of four species of Echinoidea, two with mid-bodies associated with a focalized spindle remnant, and two with neither.

The same relation between the presence and absence of a spindle remnant and the presence and absence of a mid-body may exist even in the same material, under different experimental conditions. When *Arbacia* egg-sets are run at various temperatures, those which cleave at temperatures from 10° to 25° C. show both mid-body and spindle remnant, but at 30° neither is present.

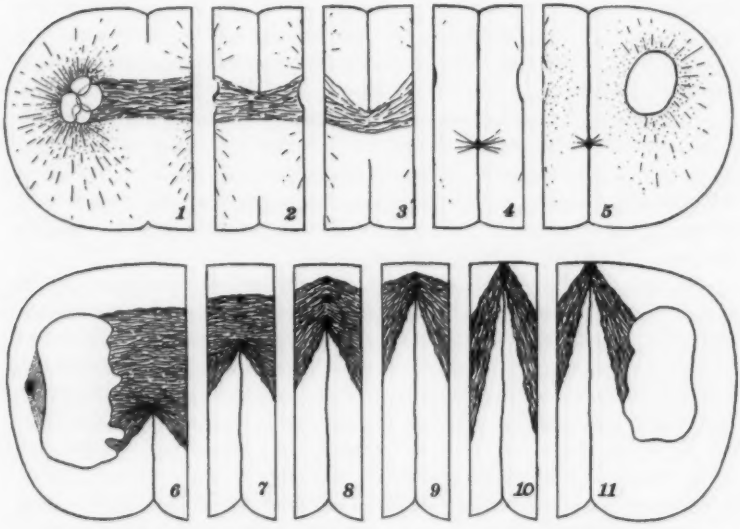
As just noted, a mid-body does not arise unless a remnant of the spindle is present when division occurs. Conversely, when a remnant is present, a mid-body is practically always formed, but a few exceptions to this are reported in the literature. For example, in the spermatogonia of *Enteroxenos* (Fig. 55), the remnant is present without the body. Occasional instances like this are not surprising in view of the fact that the behavior of mid-bodies is so variable, depending upon species, cell cycle, fixation, and depth of stain. Scarcely a single generalization can be made about them to which some exceptions cannot be found.

History of Mid-bodies

There are a number of divergent structural types of mid-bodies, and their history differs. One type is illustrated in *Arbacia* eggs. In this case first cleavage begins with the appearance of a furrow at the animal pole (Fig. 1), but no mid-body arises until the furrow has advanced far enough into the egg to impinge upon the spindle; and the body which then forms at its tip is just a faint thickening (Fig. 2). Another furrow appears at the opposite pole about a minute later, and a similar body arises at its tip when it too presses upon the spindle. Meanwhile the first furrow has advanced more deeply and its body enlarges. By the time the two furrows are ready to touch each other, a mid-body is

present at the end of each, and when contact is made these bodies join to form a dumbbell-shaped structure which undergoes no further change until it disappears when the spindle remnant fades (Figs. 3–5).

Another type is illustrated in the brain cells of *Squalus* embryos, in which division occurs from one side only. A mid-body appears at the point where the spindle is first compressed by the approaching furrow. As it advances, other bodies arise, their size and number differing from cell to cell, but the end result in all is a single body at the opposite side of the cell, where the spindle is finally focalized. It too disappears when the spindle disintegrates (Figs. 6–11).



FIGS. 1–11. Successive stages in the formation of mid-bodies. Figs. 1–5: *Arbacia* eggs at first cleavage. Figs. 6–11: brain cells of *Squalus* embryos (Fry and Robertson, 1933).

Several studies in the literature illustrate the history of other types. In erythrocytes of duck embryos (Heidenhain, 1907) a group of nondescript thickenings are aggregated into a centriole-like body (Figs. 16–20). In smooth muscle cells of *Amblystoma* (Pollister, 1932) an atypical elongate mid-body undergoes progressive condensation (Figs. 21–26). And in *Arion* eggs (Lams, 1910) slender elongate thickenings which arise at the middle of the spindle fibers are pinched together into a dense mass (Figs. 27–34).

Although these types differ as indicated, they all have in common a gradual aggregation of mid-body material associated with an increasing focalization of the spindle brought about by the division process.

The final position of the fully formed mid-body is determined by the point where the division furrows make contact. For example, in red blood cells of the duck, the furrows meet at the middle of the cell, and the mid-body lies there (Fig. 19). In *Arbacia* eggs during first cleavage one furrow arises about a minute before the other; their meeting point is therefore considerably off center, and the body lies there (Fig. 5). In *Squalus* brain cells there is only a single furrow arising at one side which presses the spindle against the opposite cell membrane, and the body lies there (Fig. 11).

In most instances mid-body and spindle remnant disappear simultaneously. This is generally true of the materials examined in the present study. It is also shown in the illustrations in the literature, where telophase figures which have both body and remnant are usually succeeded by a drawing of the next mitotic phase without either. In some cases, however, the body persists for a short time after the remnant has disappeared. This point was studied in *Arbacia* eggs at first cleavage. After division is completed, all cells have both mid-bodies and spindle remnants, but about fifteen minutes later, just before the next prophase figure makes its appearance, the remnant is doubtful or absent in about 25 per cent (count of 50), although the bodies are still present in all cells. By the time the prophase figure arises mid-bodies have, with rare exceptions, disappeared. A few cases are also found in the literature where the mid-body is shown as persisting after the spindle remnant has faded, as for example, in red blood cells of the duck (Fig. 20). In other words, if one considers the converging configuration as a whole, the focal area is obviously the most condensed part, and hence this may explain why it occasionally persists longer than the surrounding region of the converging fibers, although it may have been originally created as a result of their becoming focalized.

Configurations of Mid-bodies

The configurations which mid-bodies show during their formation, when cell division is not yet completed and spindle fibers are only partially pressed together, must be distinguished from their final structure, when the aggregation of fibers is fully accomplished. The data here presented refer only to fully formed mid-bodies; the limited number of atypical ones will be described later.

Many mid-bodies are round, smooth, and centriole-like (Figs. 11,

19, 46, 58, and 62); or their surface may be rough (Figs. 56, 57, and 60). Another common type is elongate: it may be ovoid and smooth (Fig. 47), ovoid and rough (Figs. 45 and 59), dumbbell-shaped (Figs. 5 and 61), or irregular (Figs. 48 and 49); it may lie parallel to the major axis of the spindle (Fig. 61) or at right angles to it (Figs. 5, 45, 47, 48, 49, and 54).

In addition to these common forms, other types are found occasionally. The body may be ring-shaped (Figs. 29, 36, and 37), cone-like (Figs. 30 and 52), linear in form (Fig. 26), or it may be composed of thickenings which arise at the middle of the spindle and are then aggregated loosely (Fig. 51) or tightly (Fig. 33). A specialized type is a large irregularly outlined area containing a small vesicle (Fig. 43).

In general the above types are found only when the division process has focalized the spindle to a point or to a very limited area. But associated with a specialized kind of remnant (mitosome) which is not focalized to a point, and persists as a relatively broad band of fibers, is a very different type of mid-body, composed of a row of granules (Fig. 14). In the studies examined this type was found only in spermatogonia and spermatocytes, and not in all of them. Of 15 investigations of spermatogenesis taken at random from the literature, 13 show this unusual remnant and the other two have a fully focalized remnant with a single mid-body (e.g., *Enteroxenos*, Figs. 56 and 57).

In cleaving eggs of *Arion* the mid-body is also a broad band of aggregated spindle fibers, but no granules are present (Fig. 33); and in the polar body cycles of this species there is a similar band associated with a ring-shaped mid-body (Fig. 29).

It is significant that the mid-body which is composed of a row of granules is found only in the specialized broad spindle remnants of male germ cells, whereas the other types which are usually more aggregated, are found with remnants which are focalized to a greater degree.

In some cases the very configuration of the mid-body indicates that it is focalized spindle substances. In cleaving *Arion* eggs, for example, thickenings which appear at the mid-region of the spindle are pressed together by the division furrow into a bundle, which is later bent into a U-shaped figure when the nuclei move from their former position toward the surface of the egg (Figs. 32–34). In cone-shaped bodies, the very form shows that they are spindle substances which have been pinched together (Figs. 30 and 52). And when the atypical, elongate mid-body of smooth muscle cells of *Amblystoma* makes its first appearance it is obviously a condensation of spindle materials (Fig. 22).

The other types—dots, dumbbells, ovoids, rings, lines, and vesicles—which do not appear to the eye to be focalized spindle substances,

nevertheless behave like those which do. In general, all types arise after focalization of the spindle has begun, become more aggregated as focalization is completed, and disappear as the spindle remnant disappears. A series of structural groups could be arranged, beginning with the centriole-like type, and passing by gradations to those which are clearly aggregations of spindle materials.

Relation Between Structure of Mid-bodies and Coarseness of Spindle Fibers

When mitotic figures are subjected to various experimental conditions and fixed with different reagents, mid-bodies often vary in size and shape, and the fibers vary in coarseness. The relation between the physical structure of the mid-body and the distinctness of the fixed fibers was studied in primary spermatocytes of *Romalea* and in cleaving eggs of *Arbacia*.

TABLE I

The relation between the size of the granules composing the mid-bodies in primary spermatocytes of Romalea microptera and the coarseness of the spindle fibers. In general, the coarser the fibers, the larger are the granules, but there are various exceptions to this generalization. Forty-eight modifications of the technique were used, and the depth of stain was similar in all. The table shows the percentages of each type of association of mid-body and spindle remnant.

Size of Granules Composing Mid-bodies	Coarseness of Spindle Fibers		
	Vague or Delicate	Coarse	Very Coarse
	per cent	*per cent*	*per cent*
None	8	—	3
Small (0.7 μ)	18	13	21
Medium (1.0 μ)	—	18	15
Large (1.3 μ)	—	—	4

Romalea spermatocytes were subjected to 48 experimental modifications, differing both as to environmental factors prior to coagulation and the fixatives employed. They were all stained to the same depth with Heidenhain's hæmatoxylin. Under usual conditions the mid-body is composed of a row of practically contiguous granules, each about 1 μ in diameter, and the spindle fibers have a certain degree of distinctness. The same situation obtains in spermatogonia (Fig. 14). After modifications of the technique, however, the granules may be absent, small, medium, or large, and they may vary in shape; the fibers may be vague, delicate, coarse, or very coarse.

The relation between variation in size of granules and coarseness of fibers is reported in Table I. In general, the more delicate the fibers

are, the smaller the granules. But the presence of very coarse fibers does not guarantee large granules, and those of small size are associated with fibers of all degrees of coarseness.

In *Arbacia* eggs, in the 75 experiments previously noted, the dumbbell-like mid-body is in most cases of usual length—about 1.5 μ—and the small spindle remnant is composed of delicate fibers. But in 6 of the experiments the fibers are unusually coarse and the bodies are larger. In this material too, however, there are a few exceptions: the remnant may have the usual delicate appearance and yet be associated with exceptionally large bodies, or the fibers of the remnant may be very coarse while the bodies are of usual size.

In general, therefore, the size of the mid-body is directly proportional to the coarseness of the spindle fibers, but there are exceptions. These are probably explained by the uncertainties of focalization phenomena—which is another way of saying that we do not know what the structure of the living spindle is, and we know even less about the effects of coagulation upon it, especially at points of focalization. Furthermore, when we are dealing with areas of focalization, we must also remember that slight differences in depth of stain may produce marked differences in appearance.

Variations in Mid-body Structure from One Cell Cycle to Another in the Same Species

Within the cell types of any one species—oögonia, oöcytes, spermatogonia, spermatocytes, large blastomeres, small blastomeres, and the innumerable kinds of somatic cells—there may be wide variation in mid-body structure.

In eggs of *Cerebratulus*, *Cumingia*, *Nereis*, *Chætopterus*, and *Asterias*, the mid-bodies of the first polar body figure are larger than those of the second. In *Arbacia* eggs, also, during the first three cleavages, the average length of the dumbbell-shaped bodies is successively 1.5 μ, 1.4 μ, and 1.2 μ (25 measurements in each case). And in *Cumingia* the diameter of the mid-body at first cleavage is 1.6 μ, but at the second it is 1.0 μ. In all these cases Bouin's reagent was employed. It is probable that differences in the size of the mitotic figure may explain why mid-bodies are larger in the first polar body figures than in the second, and larger in first-cleavage figures than in those following.

In *Nereis* eggs, during the first polar body cycle, the mid-body is round and relatively large, with a rough surface (Fig. 60); during the second cycle it is dumbbell-shaped and small, with a smooth surface (Fig. 61); at first cleavage, however, it is absent (Bouin's fixation). This is probably explained by the fact that a spindle remnant is

present when the polar bodies are pinched off, but not when the egg divides.

Crepidula eggs (Conklin, 1902) show striking differences in mid-body structure from cycle to cycle. In first polar body figures it is a ring, which appears as two dots when seen in cross-section (Figs. 35 and 36); in the second, it is a smaller ring with a dot in it (Fig. 37), or just a dot (Fig. 38); in first-cleavage figures it is a large area containing a vesicle (Fig. 43); in third-cleavage figures it is a small irregular blob (Fig. 45), and in fourth-cleavage figures, a minute dot (Fig. 46). The difference in size of the mid-bodies is apparently related to the size of the mitotic figures concerned. The factors which produce rings in some cells and vesicles or dots in others are not as yet understood.

Variation in the structure of mid-bodies is also exhibited in *Enteroxenos* cells in different cycles (Figs. 47–57).

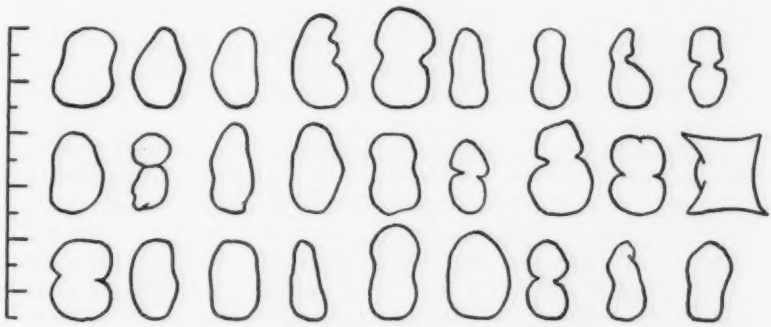


FIG. 12. Variation in mid-body structure in *Arbacia* eggs at first cleavage. Twenty-five bodies selected at random from a single slide are shown in outline and without their accompanying spindle remnants. The unit of the scale is 0.5 μ. All eggs are in late telophase just after cleavage is completed. Bouin's fixation.

Instability of Mid-body Structure

Mid-bodies vary in size and shape from cell to cell on the same slide. Outline drawings of mid-bodies in *Arbacia* eggs at first cleavage (Fig. 12) illustrate the variation in size and contour which may occur in cells at the same stage treated identically and lying side by side on the same slide. The extent of this variation is typical of the materials reported in this paper.

Mid-bodies of oögonia (Figs. 47–49) and those of the second polar body figures (Figs. 52–54) in *Enteroxenos* cells also exhibit variation in shape.

Mid-bodies vary in structure due to the use of different fixatives. In some instances a wide modification of the technique has but little effect upon the structure of the mid-body, as in *Arbacia* eggs at first cleavage (Fig. 5), but in others the effects are considerable, as in primary spermatocytes and spermatogonia of *Romalea* (Fig. 14 and Table I). As a general rule, if fixation demonstrates coarse spindle fibers, the mid-bodies are large; if the fibers are delicate, the bodies are smaller.

Mid-body structure is varied by modifications of environmental factors. In *Arbacia* eggs the size of the mid-body at first cleavage is modified by temperature. At temperatures from 25° to 20° C. the length is 1.5 μ; at 15° C. it increases to 2.8 μ; at 10° and 7.5° it again has an average length of 1.5 μ. There is, however, much greater variation at the lower temperatures than at the higher ones. (Bouin's reagent used; counts of 25 in each case.)

When *Cerebratulus* eggs cleave at 20° C., mid-bodies are present only in about 2 per cent of the cells (Boveri's picro-acetic reagent), and are less than 1 μ in diameter; but when these eggs are allowed to develop at 15° C., and are fixed in the same manner, mid-bodies over 2 μ in diameter are present in about 10 per cent of the eggs. (Counts of 50 eggs.)

Mid-bodies may be present in some cells and absent in others prepared in an identical manner. A given egg-set may be fixed under usual laboratory conditions and run up in the regular manner, yet cells on the same slide and at the same mitotic phase may show mid-bodies and remnants in some cases and neither in others. For example, *Cerebratulus* eggs, as just noted, have only 2 per cent with mid-bodies and spindle remnants at first cleavage. *Cumingia* eggs at first division show 18 per cent (Bouin's fixation), and *Arbacia* eggs show 30 per cent when dividing at 7.5° C. (Bouin's reagent). These percentages are based on counts of 50 eggs. Various similar examples could be cited.

But no matter how the experimental technique is modified, a mid-body is present only when there is a spindle remnant, with the rare exceptions previously mentioned (pp. 569 and 571). How shall we explain, then, the presence of a spindle remnant in some eggs and its absence in others which are at the same mitotic phase, the two kinds lying side by side on the same slide? The probable cause of this variation is an uncontrollable factor in the fixation process: eggs are added to the reagent in a certain amount of sea water, necessarily causing some dilution of the fixative. Each egg is coagulated within the first second after exposure to the reagent (based on a study of *Chætopterus* eggs (Fry, 1932, pp. 173–176)), but several seconds are required for the egg suspension and the fixative to mix completely.

Some of the eggs are thus coagulated by the reagent at full strength and others at various degrees of dilution. In *Chætopterus* eggs such uncontrollable dilutions of the fixative produce marked differences in the structure of astral rays and central bodies (Fry, 1932, pp. 161–167). This factor may also operate in the case of mid-bodies and their associated spindle remnants, and may explain their instability of structure, their presence in some cells and absence in others on the same slide and their differences in size and contour from cell to cell.[3]

Mid-bodies in Protozoa

Mid-bodies are usually absent in Protozoa. In the majority of species mitosis is intranuclear, the nucleus separating into two parts while the membrane remains intact. The morphological details of the process of separation vary widely: in many cases the connecting strand, prior to the final separation, is long and thin, while in others it is relatively broad; in some the spindle fibers are visible up to the time the break occurs, and in others they disappear before then. But in most species there is no special point of focalization and no mid-body. There are a few exceptions, however, where the mode of nuclear constriction aggregates the spindle fibers to a point or a limited area, and these may have mid-bodies, as in *Ceratium* (Lauterborn, 1895) and *Coccidium* (Schaudinn, 1900).

In the occasional instances where the nuclear membrane disappears and the division figure lies in the cytoplasm, a mid-body may arise as the spindle is focalized by the process of cell division, exactly as in eggs of Metazoa. This is illustrated in *Acanthocystis* (Schaudinn, 1896). But if cell division does not occur at the time when such a cytoplasmic figure is present, and the spindle is hence not focalized, there is no mid-body, as shown in *Monocystis* (Muslow, 1911).

Atypical Mid-bodies

The mid-bodies thus far described arise without exception only as spindle fibers are focalized. There are, however, several cases illustrated in the literature where thickenings appear either just prior to the process of focalization or in its complete absence.

[3] With the assistance of Dr. George Child, an attempt was made to produce mid-bodies artificially. Somewhat in advance of the time when focalization of the spindle occurs naturally, eggs of *Arbacia* and *Chætopterus* were individually constricted with a glass hair in a plane passing through the middle of the late anaphase spindle, in order to focalize it artificially. They were fixed instantaneously while the needle was still in position, and then run up individually. The experiment failed, because the protoplasm was rendered completely hyaline by the manipulation, in the plane through which the needle passed, and no structure of any kind could be seen.

In *Arion* eggs (Lams, 1910), both in polar-body and first-cleavage figures, thickenings arise at the middle of the spindle during anaphase, before division has begun. Thereafter these are aggregated in an orthodox manner into a bundle-like body when division occurs (Figs. 27–34). A similar situation is found in spermatogonia of *Blaps* (Nonidez, 1920).

In spermatocytes of *Llaveia* (Hughes-Schrader, 1931) the spindle is composed of tubes, each associated with a tetrad or a dyad. Soon after the chromosomes separate, during anaphase, the central part of each tube shrinks to a cord-like structure. At this time in some tubes, or a little in advance of the shrinking process in others, mid-bodies make their appearance—one in tubes associated with dyads, two in those with tetrads. A little later the tubes are pressed together by the division furrows and coalesce, the mid-bodies still maintaining their identity (Fig. 63).

In developing *Drosophila* eggs (Huettner, 1933) mid-bodies arise at the middle of the disintegrating spindle during telophase, in the complete absence of cytoplasmic division (Fig. 64). This also occurs in maturating eggs of *Aspidiotus* (Schrader, 1929).

These rare cases which, with the exception of *Arion*, I found only in cells of insects, do not invalidate the fact that the great majority of mid-bodies arise only in connection with the focalization of spindle fibers. No explanation can be given for these exceptions, but such behavior is not surprising in disintegrating gelatinous material.

Certain other cases reported in the literature might be regarded as atypical, but they are only additional examples of the instability of mid-bodies. For example, Buchner (1915, p. 28) illustrates in *Ascaris* eggs a mid-body of irregular shape without any spindle remnant, whereas Carnoy and Lebrun (1897, Plate II), who studied the same material and used a similar, though not identical reagent, illustrate neither body nor remnant. Such different results may be due to differences in the reagents employed, or, if the mid-body here is a staining artifact, to variations in the depth of stain. Furthermore, where Buchner shows it without an associated spindle remnant, this may be one of those occasional cases where the body persists after the remnant has disappeared.

Discussion

The Nature of Mid-bodies

The foregoing facts indicate that mid-bodies are phenomena of focalization. They do not arise in cells in which the spindle disintegrates prior to the time of division; and in those in which the spindle

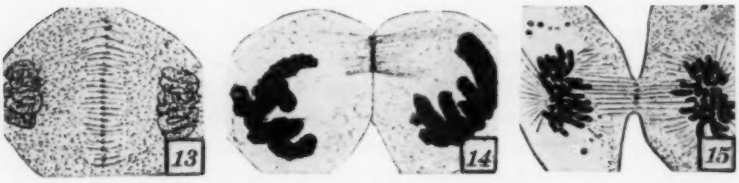
is still present at that time, mid-bodies appear only as the fibers are focalized by the process of cell division; they disappear, with rare exceptions, when such areas of focalization disintegrate. Their wide variability as to mode of formation is associated with the manner in which the spindle fibers are aggregated. Their final position in the cell is determined by the point at which the spindle remnant is pinched together by the division furrows. They often show variability in appearance from species to species, from cell cycle to cell cycle, and even from cell to cell on the same slide. They are frequently modified by differences in the fixatives employed, or variations in environmental factors prior to fixation, and such differences are usually related, with exceptions, to the size of the mitotic figure concerned, and the coarseness of the spindle fibers. In many cases their structure is unquestionably nothing but aggregated spindle substances; and it appears that such focalization phenomena may take many forms, sometimes even simulating centrioles.

Three classes of focal bodies were mentioned earlier: living focal bodies, coagulation artifact focal bodies, and staining artifact focal bodies. A number of experiments were carried out to determine to which of these categories mid-bodies belong. Some of them undoubtedly exist as a delimited structure in the coagulated cell. In these cases, when the materials are slowly destained, the result being closely observed at every stage by the use of a high power water-immersion objective, it is seen that the bodies retain the dye (Heidenhain's hæmatoxylin) to a greater extent than any other cell component, but they too finally yield it. During the destaining process they grow lighter in color but show no change in size. When the fully destained slides are run up and studied with an oil immersion objective, the bodies are still seen distinctly as refringent structures, although they are colorless. Examples of this class of focal bodies are found in the first polar-body cycle in *Nereis* (Fig. 60) and in cleaving *Arbacia* eggs (Fig. 5). Whether they existed in the living cells as minute pools of fiber material at the focal center (living focal bodies), or the living point of focalization had no such structure and the body was produced by fixation (coagulation artifact focal bodies) cannot be determined because no structure can be seen in the living spindle.

Other mid-bodies, though sharply demarked and sometimes looking exactly like centrioles, are clearly nothing but accumulations of dye at focal points. If the materials are heavily stained with Heidenhain's hæmatoxylin, the bodies are relatively large; if stained in the ordinary

manner, they are smaller. If these bodies are gradually destained, while watching the process, they do not lose their color while maintaining their characteristic size, but throughout the process they remain intensely black and become progressively smaller until they disappear. When such completely destained preparations are run up and studied in the usual way they show fibers which come to a point without the presence of a body. This is the case for example, in brain cells of *Squalus* embryos which have a centriole-like mid-body (Fig. 11).

The usual hypothesis that mid-bodies of animal cells are related in some way to the cell-plate of plant cells is not consistent with the behavior of mid-bodies as here described. The cell-plate is associated with a broad spindle which has distinct fibers in the mid-region (Fig. 13), whereas the mid-body is associated with a disintegrating spindle, and only after its fibers have been focalized. In most in-



FIGS. 13–15. Resemblances between the cell-plate of plant cells and mid-bodies of animal cells. Fig. 13: cell plate in pollen mother cells of *Fritillaria* (Strasburger, 1888). Fig. 14: fully formed mid-body in *Romalea* spermatogonia (Wilson, 1928). Fig. 15: early stage during formation of mid-body in epithelial cells of the salamander lung (Flemming, 1891).

stances, regardless of their diversity in shape, mid-bodies are single structures which do not resemble cell-plates in any way. In certain cases, however, there is enough resemblance to have given rise to the hypothesis. The mid-body which is composed of a row of granules simulates the fixed cell-plate to some extent, but the differences are marked (Figs. 13 and 14). Also, there is occasionally a brief phase during the formation of a single mid-body when several granules are present (Fig. 15) that calls to mind the cell-plate. In general, however, it seems that the mid-body and the cell-plate have nothing in common.

But regardless of the relation of the mid-body to the cell-plate, the question may be raised as to whether or not the mid-body is a true cell component playing some rôle in the process of cell division and the consequent focalization of the spindle. May it be a causative factor

and not just an effect of focalization? The findings of this study do not support such an interpretation. (1) In some cases the mid-body is only an accumulation of dye. (2) *Arbacia* eggs cleave whether mid-bodies are present (at temperatures from 10° to 25° C.) or absent (at 30° C.), indicating that they play no essential rôle. (3) This conclusion is also supported by the fact that mid-bodies are present in cleaving eggs of some species of Echinoidea but not in others. (4) And all the other data presented in this investigation make it highly improbable that we are here dealing with a cell component which plays a causative rôle.

Cells exhibit other areas of focalization—aside from central bodies which will be discussed in a moment—that behave much like mid-bodies. In his classic study of *Crepidula* eggs Conklin (1902) illustrates an unusual case. During anaphase of the second maturation division the minute centriole becomes a vacuole, while the centrosome enlarges. For a very brief period, during middle anaphase, a number of minute bodies appear, each associated with a bundle of spindle fibers (Figs. 67–69). No such bodies arise during the first polar-body cycle in this species. In *Chætopterus* eggs, however, such phenomena occur during the first maturation division but not in the second (Mead, 1898, Plate 17). In *Ascaris* eggs (Carnoy and Lebrun, 1897, Plate I) they occur in both cycles. Bělař (1928, p. 35) illustrates a somewhat similar situation in *Monocystis*.

Another possible phenomenon of focalization is shown when protoplasm and certain artificial emulsions are fixed with reagents which demonstrate foam structure (Bütschli, 1894). Minute bodies are frequently found at the points where the lines of the foam structure meet (Figs. 65–66) and these may be focal bodies.

A still different phenomenon of focalization is illustrated in telophase of the first polar body cycle of *Arion* eggs (Fig. 30). By the time the center of the old aster has disintegrated two new central bodies and asters have appeared within it. The rays of the old aster are most aggregated about this center, where they form a dark, diffuse, ring-like area of focalization.

The Significance of Mid-bodies for the Central Body Problem

If focalization of the middle of the spindle may result in the formation of structures which in some cases simulate individualized cell components, it is in order to examine the situation at the ends of the spindle, where similar focalization areas occur, of both rays and fibers, or fibers alone.

In certain cases the similarity in appearance between mid-bodies and central bodies is so marked that it compels attention. For example, Conklin (1902, p. 43)[4] notes the similarity in first-cleavage

[4] In using this quotation I have taken the liberty of substituting modern terminology (according to Wilson, 1928, p. 675) for that used in the original paper. The modern term *centriole* is used instead of the old term *centrosome* to indicate a minute, sharply demarked body; the modern term *centrosome* is used instead of the old term *sphere* to describe a larger less sharply demarked area which surrounds the centriole or exists alone; the term *central body* is a general one which includes either or both of the others.

figures of *Crepidula* eggs: "This mid-body is for all the world like a centriole with its surrounding centrosome and aster, and recalls Watasé's (1893) comparison of the mid-body to an intercellular centriole. This apparent resemblance is still further supported by the fact that the mid-body in this case becomes a hollow sphere before it finally disappears, just as the centriole does. The mid-body is surrounded by a darkly staining substance which resembles the centrosome substance" (Figs. 39–44). In short, the structural changes which occur in cleaving *Crepidula* eggs at the ends of the spindle during the early history of the mitotic figure are later repeated at the middle of the spindle when it is focalized by the division process. Furthermore, mid-bodies and central bodies are again identical in appearance at fourth cleavage, though here both are minute dots (Fig. 46). Such a similarity of appearance occurs frequently: in *Squalus* brain cells (Fig. 6), in leucocytes (Fig. 58) and connective tissue cells (Fig. 62) of the salamander, in erythrocytes of the duck (Fig. 19), in cleaving *Echinus* eggs (Fig. 59), and in many other cases.

There are numerous instances, however, where the two bodies differ completely in appearance. The sharply demarked, minute, dumbbell-like mid-body in cleaving *Arbacia* eggs (Fig. 5) bears no resemblance to the large non-demarked granular central body. The elongate mid-body in the smooth muscle cells of *Amblystoma* is unlike the typical centriole (Figs. 21 and 26). In the first maturation division of *Arion* eggs the mid-body is a band of fibers surrounded by a ring, in contrast to the minute dot-like centrioles (Fig. 29); during first cleavage, however, it is a similar band but without a ring, whereas the central body is a large area concentrically differentiated and without centrioles (Fig. 33). *Crepidula* eggs during maturation (Figs. 35–38) and *Enteroxenos* cells at various cycles (Figs. 47–57) also exhibit differences in appearance between mid-bodies and central bodies, and many other examples could be cited.

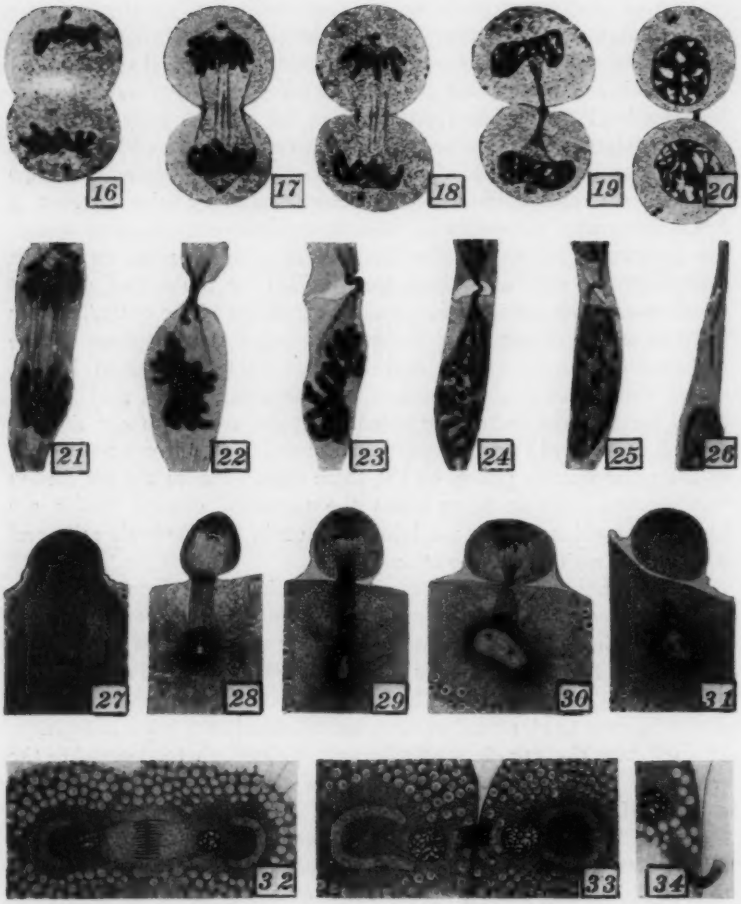
The uncertainties of focal phenomena may explain why mid-bodies and central bodies look alike in some cases and different in others, the instances of dissimilarity being somewhat more numerous. But the significant point is not whether mid-bodies and central bodies *look* alike, but whether or not they *behave* alike.

In my studies of central bodies in various cell cycles of several species, some of which were used in the present investigation of mid-bodies, I demonstrated that the structure of the central body in these cases is related to the structure of the rays or fibers. In cytasters of artificially activated *Echinarachnius* eggs (1928), where no spindle is

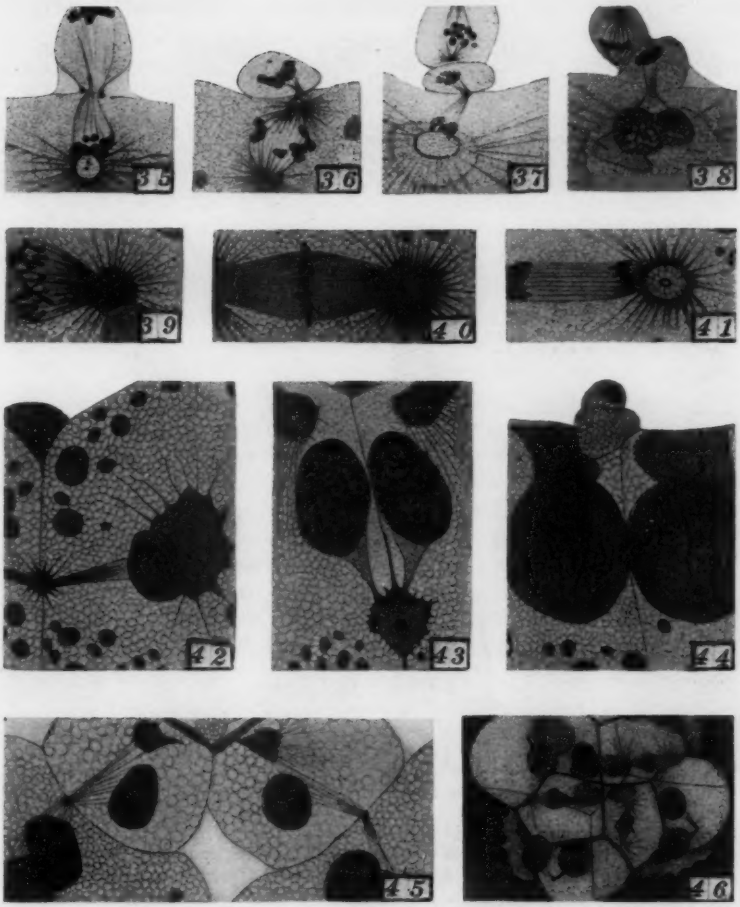
present, a central body occurs only when rays reach the center, regardless of how distinct the rays are peripherally. When a spindle is present without asters, as in *Squalus* brain cells (1933*a*), the structure is again related to the coarseness of the fibers. When the history of the central body is followed from the beginning to the end of a mitotic cycle, as it was in cleaving *Echinarachnius* eggs (1929*a*) and *Squalus* brain cells (1933*a*), it is apparent that the central body undergoes changes related to the coarseness of the converging rays and fibers, and their general configuration. The continuity of the bodies from one cell cycle to another cannot be demonstrated; they arise as areas of focalization arise, and disappear as such areas disintegrate, even though the peripheral region still has distinct rays. When, at any given mitotic phase, rays and fibers are modified by the use of different fixatives or environmental factors, the structure of the central bodies is changed, as shown in *Echinarachnius* eggs at metaphase of the first-cleavage figure (1929*b* and 1929*c*). This relationship is also shown with unusual clearness in *Chætopterus* eggs (1932 and 1933*b*). Here, furthermore, when supposedly typical centrioles are demonstrated, it is found that they differ in size and contour from cell to cell, at the same mitotic phase, on the same slide. Hence the conclusion was reached that in these cases the supposed central bodies are phenomena of focalization: staining artifacts in *Squalus* brain cells, and coagulation artifacts in the others.

The technique used in studying the mid-bodies reported in the present paper was the same as that employed in the investigations of central bodies just noted. Many fixatives were used to modify the structure of the fibers, in order to determine the effects of such modifications upon the structure of the bodies; for the same reason, cells were also subjected to abnormal environmental conditions prior to fixation. In each case the sample studied was large enough to determine the extent of structural variation, and all classes were reported and considered in arriving at the conclusion.

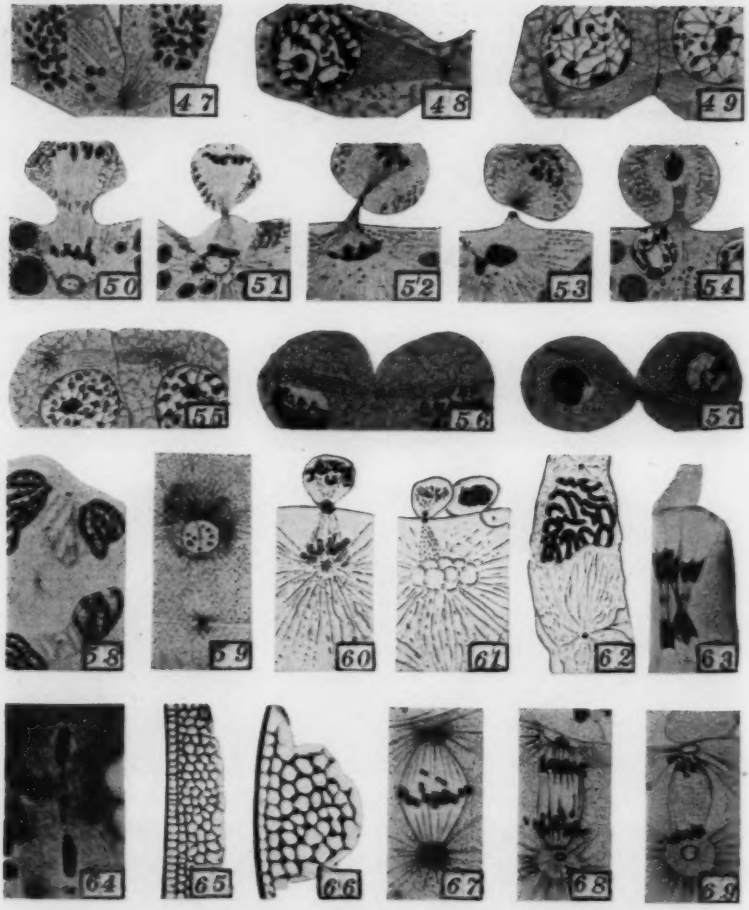
If focalization phenomena are involved in the formation of both mid-bodies and central bodies, it is rather surprising that in mitotic figures which have asters, those bodies occurring at the middle of the spindle where only fibers are present, should ever look like those occurring at the spindle-ends where both fibers and rays are concerned. But, as previously mentioned, in some cases they may be identical in appearance, and in others completely dissimilar. At present it is as impossible to explain this fact as it is to explain why, for example, the mid-bodies of *Crepidula* eggs are ring-like during polar body formation, but vesicle-like at first cleavage, and centriole-like at fourth cleavage



FIGS. 16–34. Successive stages in the formation of mid-bodies. Figs. 16–20: erythrocytes of duck embryos (Heidenhain, 1907). Figs. 21–26: non-striated muscle cells of *Amblystoma* larvae (Pollister, 1932). Figs. 27–34: first polar bodies and first cleavage in *Arion* eggs (Lams, 1910).



FIGS. 35–46. Mid-body structure and central body structure in various mitotic cycles of *Crepidula* eggs (Conklin, 1902). Figs. 35–36: first polar bodies. Figs. 37–38: second polar bodies. Figs. 39–44: first cleavage. Fig. 45: third cleavage. Fig. 46: fourth cleavage.



FIGS. 47–69. Mid-bodies in various mitotic cycles of *Enteroxenos* cells (Bonnevie, 1906). Figs. 47–49: oögonia. Figs. 50–51: first polar bodies. Figs. 52–54: second polar bodies. Fig. 55: spermatogonia. Fig. 56: primary spermatocytes. Fig. 57: secondary spermatocytes. Mid-bodies of various cells. Fig. 58: salamander leucocytes (Bělař, 1928). Fig. 59: cleaving *Echinus* eggs (Boveri, 1901). Figs. 60–61: first and second polar-body figures of *Nereis* eggs (original). Fig. 62: connective tissue cells of the salamander lung (Flemming, 1891). Fig. 63: primary spermatocytes of *Llaveia* (Hughes-Schrader, 1931). Fig. 64: developing *Drosophila* eggs (Huettner, 1933). Bodies occurring at the junction of the walls of alveoli. Fig. 65: fixed eggs of *Sphaerechinus* (Bütschli, 1894). Fig. 66: artificial emulsion of olive oil and NaCl (Bütschli, 1894). Multiple bodies, in addition to central bodies, occurring at spindle-ends during middle anaphase in the second maturation division of *Crepidula* eggs (Conklin, 1902). Fig. 67: early anaphase without bodies. Fig. 68: middle anaphase with bodies. Fig. 69: late anaphase without them.

(Figs. 35–46). If, however, the focalization of fibers or rays may cause the production of focal bodies, it is not surprising that there is variation in their structure according to the size of the configuration as a whole and the coarseness of the converging fibers. But our knowledge of the physical chemistry of such systems is not sufficient for us to explain the structural diversity in focal bodies, from species to species, and cell cycle to cell cycle, in mitotic figures of the same size and distinctness.

If the conclusion of the present study is valid, we cannot accept the presence of a sharply demarked body in an area of focalization—whether at the middle or the ends of the spindle—as evidence that we have demonstrated a true cell component. This is, however, the generally accepted practice. Structures which appear to the eye to be as individualized as chromosomes or plastids may nevertheless be merely ephemeral transient phenomena of focalization—sometimes nothing but focal accumulations of dye, as in both mid-bodies and centrioles in brain cells of *Squalus* (Fig. 6) (Fry and Robertson, 1933). The eye sees such a structure, and the mind accepts it on the basis of its appearance and attempts to imagine its function.

The fact that bodies do not arise at the middle of the spindle until it is focalized by the division furrows should make us alert to possible pitfalls—errors in interpretation—when structures are found at points where fibers or rays or both converge. In this connection it is significant that bodies are not present at the ends of anastral spindles unless the tips are sharply focalized. The blunt, anastral spindles found in some oöcytes and spermatocytes do not have them (with possibly a few exceptions in the latter when centriole-blepharoplasts are present).

If the conclusion of this investigation is correct, the current central body hypothesis must be reevaluated. Focal bodies have probably been confused with such cell components as centriole-blepharoplasts of male germ cells, diplosomes of vertebrate cells, and similar structures of Protozoa and other cell types, which for the most part are concerned with the formation of axial filaments and flagella, no matter what their rôle in mitosis. The great majority of cell types have no such components, and it is yet to be ascertained to what an extent their supposed central bodies are actually artifacts of focalization.[5]

[5] The data of the present study suggest that the spindle, despite its homogeneous appearance in the living cell, has some kind of linear organization. The very fact that bodies appear in the plane where the division furrow exerts pressure on the spindle indicates that there are differentiated materials there. If the spindle were actually homogeneous, it is probable that its materials would flow to one side or the other when it is pinched together, in which case no focalization bodies would be formed.

Resumé

Mid-bodies were studied in various cell cycles in a number of species; many fixatives were used and cells were subjected to different environmental modifications prior to fixation, to modify the structure of both bodies and fibers in order that the relation between them might be analyzed.

Mid-bodies are found only in cells in which the spindle is still present when division occurs; they are absent in the numerous cases where the spindle disappears before that time. They arise only as the spindle fibers are gradually brought to a focus by the advancing division furrows; they usually disappear simultaneously with the spindle remnant. Their final position is determined by the point where the division furrows meet. There are many structural types: centriole-like dots, blobs, ovoids, dumbbells, rings, cones, lines, rows of dots, and other configurations. Some are obviously nothing but aggregated spindle materials, whereas others look like individualized cell components. Different structural types may occur in the same species in successive cell cycles. They may vary in size and contour from cell to cell on the same slide. They are generally unstable and easily modified by the use of various environmental factors or different fixatives. Such structural modifications are usually related to the coarseness of the fibers.

Mid-bodies are generally regarded as a vestigial homolog of the cell-plate of plants. The present study, however, interprets them as phenomena of focalization or "focal bodies." It is suggested that these are produced as the result of the concentration of spindle or aster materials at points where they are focalized. In some cases mid-bodies are only an accumulation of dye at the focal area; in others they are probably produced by the process of fixation.

If bodies which look like typical cell components can be formed at the middle of the spindle, as the result of the focalization of its materials brought about by the advancing division furrows, similar phenomena may occur at the areas of focalization at the spindle ends where both fibers and rays may converge. Previous studies of central bodies, in some of the same cells used for the present study of mid-bodies, indicate that they too are produced as a result of the focalization of spindle and aster materials. It is, therefore, suggested that in the formulation and development of the current central body hypothesis, focal bodies may have been confused with true cell components such as centriole-blepharoplasts, diplosomes, and similar structures.

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A PHYSIOLOGICAL AND HISTOLOGICAL STUDY OF THE FRONTAL CORTEX OF THE SEAL (PHOCA VITULINA) [1]

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The so-called "motor areas" of the cortex of the cat, dog and bear have been investigated recently with the combined methods of electrical stimulation and histological examination (Langworthy, 1928; Smith, 1933, 1935, etc.). The general pattern, both functional and anatomical, of these three fissiped carnivores is quite similar, although minor variations are distinct. It was therefore of interest to make a comparative study of the harbor seal (*Phoca vitulina*), a pinniped carnivore which is functionally adapted to an aquatic existence and in which the trunk and extremities are very considerably modified (Howell, 1928).

I should like to express my thanks to the Superintendent, Mr. Thomas H. Dorr, and his assistants, particularly Mr. E. C. Barter, at the Boothbay Harbor Station of the United States Bureau of Fisheries, for obtaining the animals and assisting in handling them.

METHODS

Experiments were successfully completed on 6 seals approximately 4 to 5 months old. The animals were in excellent condition and weighed between 18 and 25 kgm. Satisfactory anæsthesia was obtained with Dial fluid (Ciba) 0.3 cc. per kgm. intraperitoneally, supplemented by a few whiffs of ether during the opening of the skull. It was found preferable to keep the body of the seal submerged in a tank of water during the early stages in order to assist respiration.[2] The thin skull was readily removed with trephine and rongeurs. Since the electrically excitable cortex was located behind the eye, the latter was enucleated and the orbital roof removed in

[1] The seals were obtained and the physiological observations made at the Boothbay Harbor Station of the U. S. Bureau of Fisheries.

[2] When on land the seal normally breathes rhythmically and regularly. Five to ten minutes after the injection of the dial, respirations became intermittent. Four to five deep breaths were taken, ending in inspiration, following which the nostrils were tightly closed and the breath held for 15 to 40 seconds, the cycle being then repeated. The administration of ether by a cone during three or four of these cycles resulted in regular, slow deep respiration.

the later experiments. The rectal temperature of the animals was kept between 37° and 40° C. by pouring cold sea water over them occasionally.

Stimulation was bipolar by means of a pair of silver-silver chloride electrodes with an interpolar distance of 2 mm. Current was supplied by the 60 cycle a.c. line through a potentiometer. The effective voltage varied in different animals from ½ to 5 volts. For single

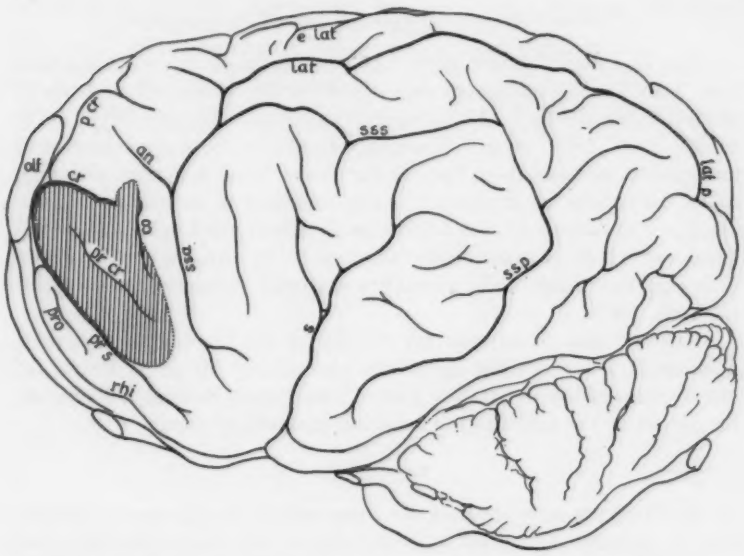


FIG. 1. Outline drawing of a lateral view of the brain of seal 5. × 1.5. The position of the electrically excitable cortex is indicated by the shaded area. *an*, S. ansatus. *co*, S. coronalis. *cr*, S. cruciatus. *e lat*, S. endolateralis. *lat*, S. lateralis. *lat p*, S. lateralis posterior. *olf*, Bulbus olfactorius. *p cr*, S. postcruciatus. *pr cr*, S. precruciatus. *pr s*, S. presylvius. *pro*, S. proreus. *rhi*, Fissura rhinalis. *s*, S. pseudosylvius. *ssa*, *ssp*, *sss*, S. suprasylvius anterior, posterior and superior.

shocks a Harvard inductorium, with 3 volts in the primary circuit and the secondary coil set at 7 to 9 cm., was employed. Between observations the cortex was kept moist by application of cotton pledgets wet with warm Ringer's solution.

At the end of the experiment the brains were removed and preserved in formalin. Serial sections in the sagittal plane were cut at 35 μ of the frontal poles of the left hemispheres of seals 3 and 4, and

in the horizontal plane of the right hemisphere of seal 4. Every twentieth section was stained with thionine and mounted.

Results

In conformity with the rounded shape of the skull, the high position of the nostrils and the microsmatic habits of the seal, the brain tends to a more spherical form than in the terrestrial carnivores.

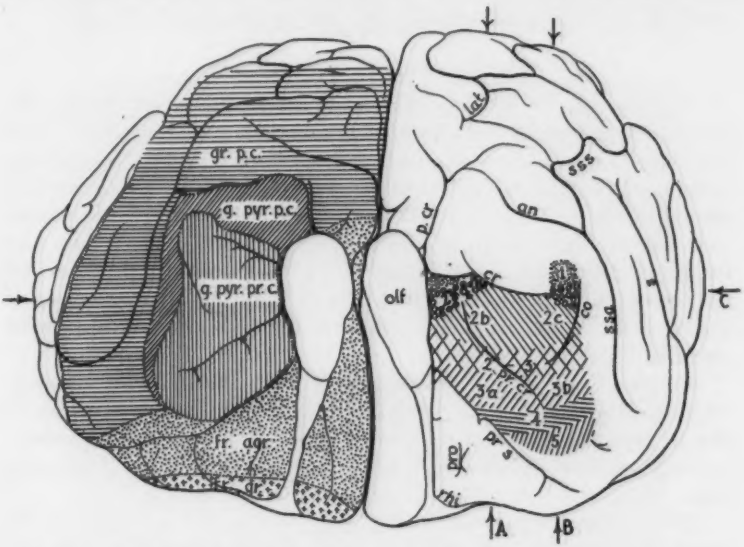


FIG. 2. Outline drawing of a frontal view of the brain of seal 4. × 1.5. The sulci are labelled as in Fig. 1.

The electrically excitable cortex is indicated on the left hemisphere. The numbers are referred to in the text.

The histological areas are indicated on the right hemisphere. *fr. agr*, Area frontalis agranularis. *fr. gr*, Area frontalis granularis. *g pyr p c*, Area gigantopyramidalis postcentralis. *g pyr pr c*, Area gigantopyramidalis precentralis. *gr p c*, Area granularis postcentralis (the caudal boundary of this area is not defined in the figure).

The general pattern of the sulci, however, was found to be similar, as illustrated in Figs. 1 and 2. A number of additional shallow sulci were present, which resulted in a reduplication and subdivision of the gyri, a condition more pronounced in other aquatic mammals (*cf.* Langworthy, 1932, 1935). Certain features in which the frontal lobe differed from that of other carnivores may be noted. The sulcus cruciatus was relatively small and was quite shallow laterally, but

medially became deeper and, behind the olfactory bulb, ran into the well-developed sulcus postcruciatus. The sulcus ansatus was well marked and entered the S. suprasylvius anterior. In some instances it extended medially as a shallow groove into the S. postcruciatus, but in no case into the S. lateralis. The S. coronalis was very shallow, appearing merely as a slight depression in some of the hemispheres. In one case it extended ventrally as far as the S. precruciatus, which was also shallow. The large Gyrus proreus was divided into three parts by the S. proreus and the thin olfactory stalk.

Physiological Observations

The boundaries of the electrically excitable cortex under the present experimental conditions were as follows. The posterior limit was defined by the S. cruciatus. Lateral to this a small area giving movements of the tail extended onto the G. sigmoideus posterior, medial to the S. coronalis. The medial and ventromedial boundaries were sharply defined at the lateral margin of the olfactory bulb and by the S. presylvius. The lateral and ventro-lateral boundaries did not follow the superficial markings except along the posterior third of the S. coronalis, but extended well onto the G. suprasylvius anterior (see Fig. 2).

In two of the animals movements of the hind limb were obtained by stimulation of high intensity (10 volts) from the posterior lip of the S. cruciatus. In no other case, however, were striped muscle responses evoked by superficial stimulation outside of the designated area.

The motor cortex was readily subdivided, on the basis of the movements evoked, into the following areas: (1) tail area, (2) tail and hind-flipper area, (3) fore-flipper area, (4) neck area, and (5) face area. These are correspondingly numbered in Fig. 2.

The tail area (1) was small and sharply defined. The only movement obtained consisted of elevation and deflection of the tail to the contralateral side.

Explanation of Figures 3–6

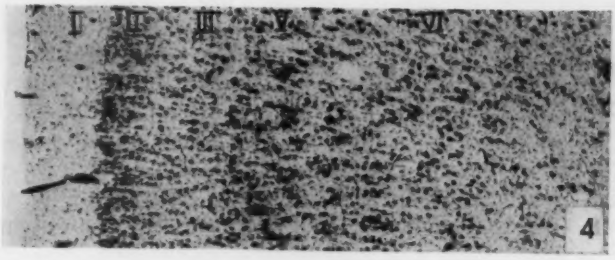
Figs. 3, 4, 5 and 6. Microphotographs of portions of a sagittal section of the left hemisphere of seal 4 in the plane indicated by arrow *A* in Fig. 2. Thionine stain. 35 μ. × 35.

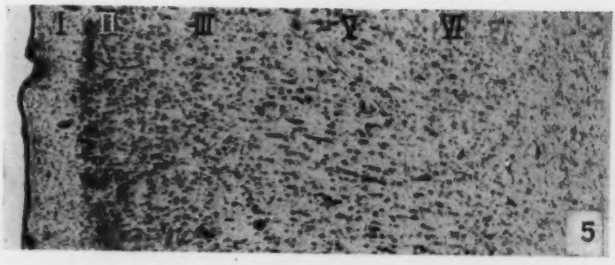
3. Area gigantopyramidalis precentralis, caudal to the S. precruciatus (hind-flipper area).

4. Area gigantopyramidalis precentralis, rostral to the S. precruciatus (fore-flipper area).

5. Area frontalis agranularis, rostral to the S. presylvius.

6. Area granularis postcentralis, through the S. ansatus.





FIGURES 3–6

The tail and hind-flipper area (*2a*, *2b*, *2c*) was the most extensive and merged into the fore-flipper area (*3a*, *3b*) in a zone (2–3) from which various combinations of movements of the contralateral fore-flipper, both hind-flippers and tail were evoked. From *2a* the tail and contralateral hind-flipper were elevated and deflected contralaterally. From *2b* and *c* similar movements resulted of the tail and both hind-flippers, together with ventral arching of the back so as to raise the hindquarters of the animal off the table. From *b* the flippers were flexed at the digits, whereas from *c* they were extended.

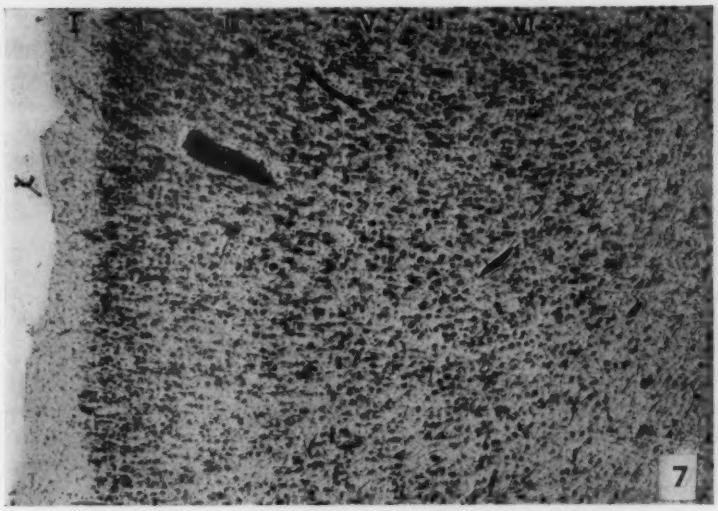
The line of demarcation between the fore-flipper and neck areas was much sharper than that between the fore- and hind-flipper areas. Movements of the contralateral, but not of the ipsilateral, fore-flipper were obtained. From *3a* there was extension of the digits, moderate extension at the elbow and adduction at the shoulder. From *3b* there was flexion of the digits and elbow with abduction at the shoulder.

The boundaries of the neck area (4) were moderately well defined. Movements of the shoulder girdle, drawing the shoulder forward, and of the neck, with deviation of the head to the contralateral side, were obtained either alone or in combination. The shoulder was localized posterior to the neck.

The face area (5) was somewhat larger than the tail area, but much smaller than those for the flippers. Retraction of the contralateral angle of the mouth and closure of the contralateral eye, usually separately, occasionally together, were the only movements elicited.

The boundaries between areas 3 and 4, and 4 and 5 could be defined within approximately 1 mm. in a single series of stimulations, but there was a shift of the boundary of as much as 2 mm. backwards or forwards in successive series of observations depending on whether the previous stimuli had been applied in front or behind respectively. Similarly, the predominating movements evoked from the zone 2–3 depended on whether the preceding stimulation had been of the hind- or of the fore-flipper areas.

The most excitable area of the motor cortex was found in the central region of the hind-flipper area (the region of the largest giganto-pyramidal cells, see below). Here, in four of the seals, an intensity of 0.5 volts with a.c. stimulation evoked strong movements and in one case a single break-shock (Harvard inductorium, 7 cm.) resulted in a jerky contraction of the contralateral hind-flipper. Epileptiform after-discharge of varying duration was regularly elicited by a.c. stimulation of 3–5 volts for 10 to 20 seconds from any of the areas, but was most marked from areas 2 and 3.



FIGS. 7 and 8. Microphotographs of portions of sagittal sections of the left hemisphere of seal 4. Thionine stain. 35 μ. $\times$ 35.

7. Transition from the Area gigantopyramidalis precentralis (face area) to the Area frontalis agranularis, the lower border of the electrically excitable area in the plane of arrow *B*, Fig. 2.

8. Area gigantopyramidalis postcentralis and the transition to Area granularis postcentralis above, just rostral to the S. ansatus in the plane of arrow *A*, Fig. 2.

Histological Observations

The exposed cortex of the frontal lobes was well developed and the several cell layers clearly defined. The average thickness was approximately 2 mm. In the depths of the sulci, however, it became much thinner, at the expense of the lower layers, and less well differentiated. This was particularly marked in the cruciate, precruciate and presylvian sulci.

The histological areas conformed in general to the pattern and structure described by Smith (1935) in the dog. Their extent on the exposed surface of the frontal lobe is shown in Fig. 2. Certain characteristics of the classical six-cell layers of the several areas, as seen in sagittal and horizontal sections, may be summarized as below.

Area gigantopyramidalis precentralis.—(Figs. 3, 4, 7, 9 and 10.) Layer I was better developed than in the other areas, but Layer II was relatively thinner and less dense, with many small to medium-sized pyramidal cells. Layer III was very broad, with numerous medium-sized pyramidal cells. Layer IV, absent. Layer V contained typical giant pyramidal cells which tended to be arranged in groups between fiber bundles. This layer showed more variation than did the others in different parts of the area. The cells were largest in the upper, central region (Fig. 1) (the central portion of the hind-flipper area), becoming smaller toward the periphery (Figs. 7, 9, 10) (including the tail, neck and face areas). There was a rather abrupt change from the posterior to the anterior lip of the precruciate sulcus (*cf.* Fig. 3 with Fig. 4), but elsewhere the transition was gradual. These cells were also considerably smaller around the sulci, particularly in the lower half of the cruciate sulcus, although a narrow band of gigantopyramidal cells again appeared in the posterior lip of this sulcus. Layer VI was broad, with numerous small, fusiform cells.

The myeloarchitecture was prominent throughout the area and the cells of layers III, V and VI appeared to be grouped between radiating bundles of fibers. Around the bottoms of the sulci, however, the orientation of the cells seemed to be determined by U fibers.

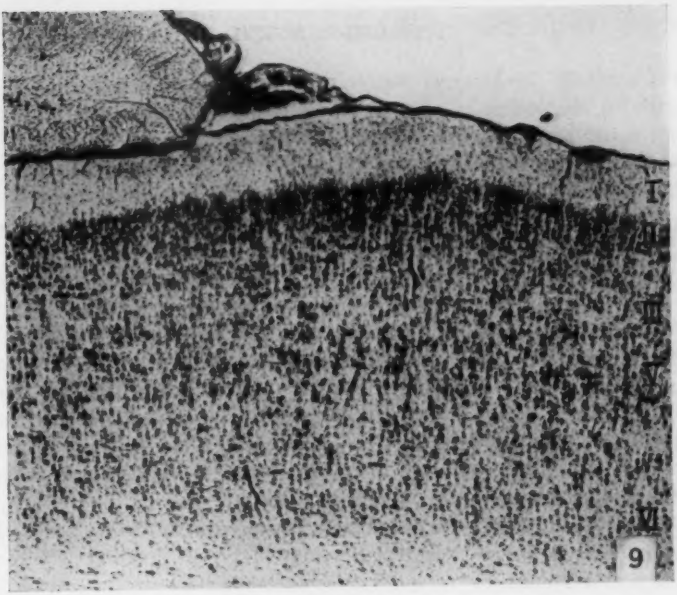
The boundary of the Area gigantopyramidalis precentralis and the

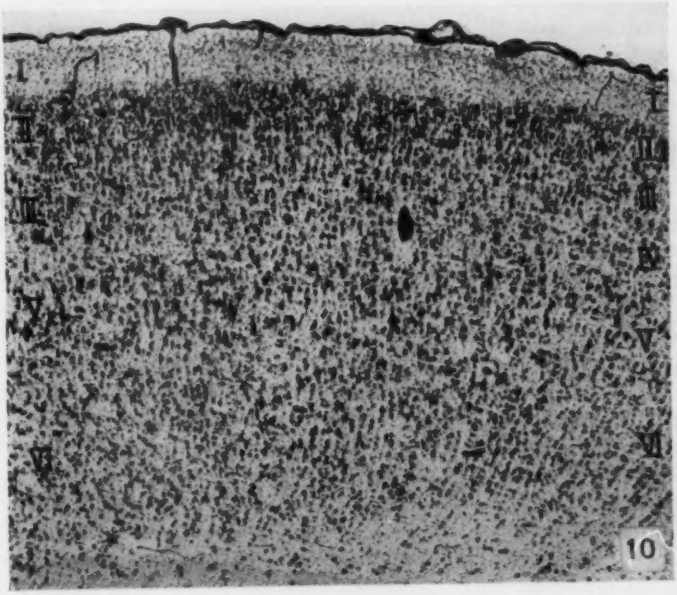
Explanation of Figures 9 and 10

Figs. 9 and 10. Microphotographs of portions of a horizontal section through the right hemisphere of the brain of seal 4 in the plane indicated by arrow *C*, Fig. 2. Thionine stain. 35 μ. × 35.

9. Transition from the Area gigantopyramidalis precentralis (right), to the Area frontalis agranularis (left), at the lateral margin of the Bulbus olfactorius (upper left).

10. Transition from the Area gigantopyramidalis precentralis (extreme left) through the Area gigantopyramidalis postcentralis (center) to the Area granularis postcentralis (extreme right) between the S. coronalis and the S. suprasylvius anterior.





FIGURES 9 AND 10

Area frontalis agranularis was quite sharp (Figs. 7 and 9). The transition, however, to the Area gigantopyramidalis postcentralis (Fig. 8) and to the granular cortex of the G. suprasylvius anterior was very gradual (Fig. 10).

Area frontalis agranularis.—(Figs. 5 and 7.) In comparison with the precentral gigantopyramidal cortex this area showed the following characteristics. Layer I was approximately half as thick. Layer II contained more cells, but with a smaller proportion of pyramidal forms. Layer III also showed an increase in number of cells. Layer IV was absent. Layer V varied in thickness at different regions and the cells varied in size from slightly smaller to slightly larger than those of layer III. Occasional cells contained heavily staining Nissl substance, but the majority were relatively pale. Layer VI was similar to that in the area gigantopyramidalis. The myeloarchitecture was less prominent, although in places the radiating arrangement of cells between fiber bundles was noticeable.

This type of cortex extended over a wide area, including most of the G. proreus and the ventral portions of the G. genualis where a transition to a granular type of cortex occurred posteriorly. Anterior to this area, between it and the undifferentiated cortex of the basal olfactory areas, was the Area frontalis granularis. The latter was poorly defined and small in extent. It was somewhat thicker, but the cell types were similar with the addition of the granular cells of layer IV.

Area gigantopyramidalis postcentralis.—(Figs. 8 and 10.) The following changes occurred through the transition zone between this and the Area gigantopyramidalis precentralis. Layer I was reduced to about one-third as thick. Layer II increased in density and breadth, most of the cells being rounded in form. Layer III was considerably reduced and the cells became smaller. Layer IV appeared as a narrow, ill-defined band of granular cells. The giant pyramidal cells of layer V persisted, but greatly reduced in numbers and size. Layer VI showed little change. The radiating bundles of fibers through the lower layers remained prominent.

This area covered the greater part of the exposed surface of the G. sigmoideus posterior, the transition to the Area granularis postcentralis being sharply defined at the postcruciate and ansate sulci (Fig. 9). A similar, though narrower, zone extended round the lateral border of the Area gigantopyramidalis precentralis, between it and the granular cortex of the G. suprasylvius anterior (Fig. 10).

Area granularis postcentralis.—(Figs. 6 and 8.) This area differed strikingly from those described above. Layer I was narrow. Layer II was broad and dense, the cells being mainly small and rounded. It appeared to fuse with layer IV, which contained similar cells.

Layer III showed as a band of scattered, small to medium-sized, pyramidal cells amongst the granular cells at the junction of layers II and IV. Layer V was sharply defined, consisting chiefly of fibers, with a few cells of the small, pyramidal type. Layer VI was well developed and for the most part contained rounded cells with occasional fusiform and triangular elements.

The decrease in thickness of this type of cortex round the base of the sulci was much less marked than in the other areas and was mostly due to diminution in layer VI.

This area extended caudally over the G. lateralis and rostrolaterally over the G. suprasylvius anterior. In the latter location layer V was better developed than it was caudally. Laterally it was bounded by an area in the anterior wall of the S. suprasylvius anterior which showed a poorly developed layer III, a narrow, irregular layer IV, and sparsely scattered, but very large, pyramidal cells in layer V. Rostral to the Area gigantopyramidalis precentralis the transition to the Area frontalis agranularis was relatively sharp.

Discussion

The present experiments were performed under a single form of anæsthesia and on animals otherwise normal. Because of these limitations it is obvious that conclusions with regard to correlations between so-called electrical excitability and the cytoarchitecture of the different cortical areas are of little or no significance (*cf.* Tower, 1936; Rioch and Rosenblueth, 1935). It is of interest, however, that the lowest threshold was found in the region of the largest Betz cells; and the observation that a strong movement could be evoked by a single shock in this area is unusual in carnivores under dial anæsthesia.

The extent and differentiation of the Area gigantopyramidalis, on the basis of both the physiological and anatomical observations, compare favorably with these features in other carnivores as observed by the present author under similar conditions of anæsthesia (dog and cat) and as described in the literature (*cf.* Langworthy, 1928; Smith, 1933, 1935, etc.). In contrast with this high degree of development of the central representation stands the apparently simpler and less differentiated form of the extremities of the seal. It may be concluded that the extent of central representation is to be correlated with function and not with form. Further evidence for this hypothesis is furnished by the following considerations. In the terrestrial carnivores electrical stimulation of the cortex evokes movements of the extremities which resemble certain phases of the normal activity of the animal when initiating locomotion, feeding, seizing prey, etc. In the seal, however, the movements of the trunk and extremities

elicited from the cortex resembled parts of the normal swimming actions, which are finely and swiftly executed, and not the clumsy movements of locomotion of that animal on land.

The rostral position of the gigantopyramidal area and the shallowness of the S. cruciatus may be due in part to the relatively small size of the olfactory brain, allowing more room for superficial development of the frontal lobes of the hemispheres.

The cerebellum, particularly the lateral lobes, and the pons were found to be very large in the seal as compared with the dog and cat, resembling the development of these structures in other aquatic mammals (Langworthy, 1932, 1935). This is probably to be correlated with the wide extent of the Area frontalis agranularis. The teleological explanation which suggests itself is the necessity for particularly fine coördination of movements controlling the position of the animal in three-dimensional space.

Summary

The exposed cortex of the frontal lobes of the left hemispheres of six young seals (Phoca vitulina) under dial anæsthesia was stimulated electrically. Movements of the tail, both hind-flippers and the contralateral fore-flipper, neck and face were evoked. The cortical localization is charted in Fig. 2.

Histological examination (thionine stain) of three frontal lobes revealed a well-developed cortex divisible into cytoarchitectural areas structurally similar to those in other carnivores, but differing in their extent and their relation to the sulci (Fig. 2).

On the basis of a comparison of the seal with the terrestrial carnivores it is concluded that the degree of differentiation of the cortical representation is to be correlated with function and not with form.

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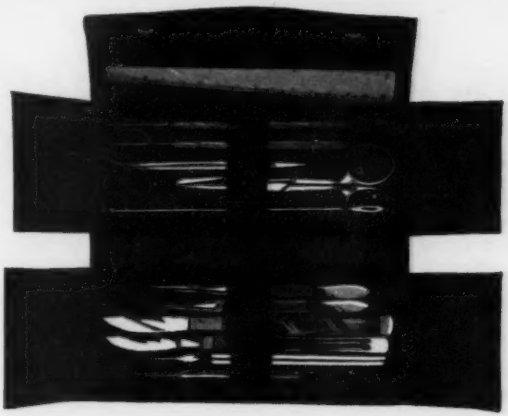

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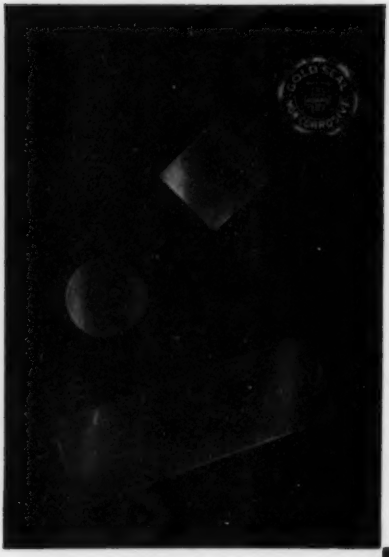

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